	L #	Hits	Search Text	DBs	Time Stamp
1	L1	215	venom near4 (protease$1 or metalloproteinase$1 or metalloprotease$1)	USPAT; US-PGPUB	2003/06/09 12:01
2	L2	1544	cobra	USPAT; US-PGPUB	2003/06/09 11:56
3	L3	16	1 same 2	USPAT; US-PGPUB	2003/06/09 12:01
4	L4	846	psgl or (p adj selectin)	USPAT; US-PGPUB	2003/06/09 12:01
5	L5	3	1 same 4	USPAT; US-PGPUB	2003/06/09 12:01
6	L6	78	(protease$1 or metalloproteinase$1 or metalloprotease$1) same 4	USPAT; US-PGPUB	2003/06/09 12:02
7	L7	11	1 and 6	USPAT; US-PGPUB	2003/06/09 12:02
8	L8	5	mocarhagin	USPAT; US-PGPUB	2003/06/09 12:02
9	L9	25	3 or 7 or 8	USPAT; US-PGPUB	2003/06/09 12:03

PGPUB-DOCUMENT-NUMBER: 20030040607

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030040607 A1

TITLE: Hematopoietic cell E-selection/L-selectin ligand polypeptides and methods of use thereof

PUBLICATION-DATE: February 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sackstein, Robert	Sudbury	MA	US	

APPL-NO: 10/ 042421

DATE FILED: October 18, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60240987 20001018 US

non-provisional-of-provisional 60297474 20010611 US

US-CL-CURRENT: 530/395, 435/320.1 , 435/325 , 435/69.1 , 536/23.5

ABSTRACT:

The invention feature methods and compositions for treating hematopoietic disorders, imflammatory conditions, and cancer and providing stem cell therapy in a mammal.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Ser. No. 60/240,987, filed, Oct. 18, 2000 and U.S. Ser. No. 60/297,474, filed, Jun. 11, 2001 which are incorporated herein by reference in their entireties.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (32):

[0053] FIG. 13B is a chart showing CHO-P cell rolling interactions on KG1a and HL60 cells that were measured over a similar shear stress range and were eliminated in the presence of EDTA and prevented by pretreating KG1a and HL60 cells with **mocarhagin** (10 .mu.g/ml).

Detail Description Paragraph - DETX (176):

[0213] OSGE was purchased from Accurate Chemicals, Westbury, N.Y., and Vibrio cholera neuraminidase and N-glycosidase-F was obtained from Roche Molecular Biochemicals, Indianapolis, Ind. **Cobra venom metalloprotease, mocarhagin** (Spertini, O., Cordey, A. S., Monai, N., Giuffre, L. and Schapira, M. (1996) J. Cell Biol. 135(2), 523-531; De Luca, M., Dunlop, L. C., Andrews, R. K., Flannery, J. V., Ettling, R., Cumming, D. A., Veldman G. M. and Berndt, M. C. (1995) J. Biol. Chem. 270(45), 26734-26737), was a gift from Dr. Ray Camphausen (Genetics Institute, Cambridge, Mass.). The metabolic inhibitor, tunicamycin, and all other chemicals were purchased from Sigma, Inc. (St. Louis, Mo.).

Detail Description Paragraph - DETX (183):

[0220] Using the parallel-plate flow chamber under defined shear stress conditions, L-selectin-mediated adhesive interactions between human HCs and L-selectin naturally expressed on leukocytes (awrence, M. B., McIntire, L. V. and Eskin, S. G. (1987) Blood 70(5), 1284-1290) was studied. Leukocyte tethering and rolling on human HC monolayers was visualized by video microscopy in real time using the parallel-plate flow chamber prepared in the following manner. Prior to experimentation, leukocytes were washed twice in HBSS and then suspended at 10.sup.7/ml in HBSS/10 mM HEPES/2 mMCaCl.sub.2 (H/H/Ca.sup.++). Negative control groups were prepared by treating cells with PMA (50 ng/ml H/H/Ca.sup.++ for 1 hr at 37.degree. C.) to induce the cleavage of L-selectin from the cell surface, by pretreating with moAb HRL-1 (10 g/ml) to block L-selectin binding, or by incubating with 5 mM EDTA to chelate Ca.sup.++ required for L-selectin binding. To prepare human HC monolayers (100% confluent), suspensions of HCs (KGla, HL60, RPMI 8402, K562) at 2.times.10.sup.6/ml RPMI-1640 without Na+ Bicarbonate/2% FBS were seeded in 6-well plates at 5.times.10.sup.6/well, centrifuged to layer cells then fixed in 3% glutaraldehyde. Reactive aldehyde groups were blocked in 0.2M lysine, and plated cells were suspended H/H/Ca.sup.++. To assess the dependence of binding by sialic acid residues on L-selectin ligands, cells were pretreated with Vibrio cholerae neuraminidase (0.1/ml H/H/Ca.sup.++ for 1 hr at 37.degree. C.). To examine the contribution of sialomucins (including PSGL-1) and PSGL-1 alone, OSGE (60 .mu.g/ml H/H/Ca.sup.++ for 1 hr at 37.degree. C.) or **mocarhagin** (10 .mu.g/ml for 20 min at 37.degree. C.) treatments were performed, respectively. Furthermore, since HCELL is expressed on KG1a cells and sialylated N-glycosylations on HCELL are critical for L-selectin ligand activity (Sackstein, R. and Dimitroff, C. J. (2000) Blood 96,2765-2774) the contribution of HCELL on KG1a cells was distinguished by first cleaving all of the sialic acid residues from the cell surface with Vibrio cholerae neuraminidase (0.1U/ml for 1 hr at 37.degree. C.) and then incubating the cells with a metabolic inhibitor of N-glycosylation, tunicamycin (15 .mu.g/ml for 24 hr at 37.degree. C., 5% CO.sub.2), to prevent de novo synthesis of N-glycans (i.e., HECA-452 epitopes on CD44/HCELL). Neuraminidase pretreatment removed all of the residual HCELL activity from the cell surface, and therefore, this treatment approach allowed for the assessment of newly synthesized HCELL on the cell surface. HC cytospin preparations were prepared in multi-well plates as described above. The parallel-plate flow chamber was placed on top of the cell monolayers and leukocytes were perfused into the chamber. After allowing the leukocytes to contact the cell monolayers at a shear stress of 0.5 dynes/cm.sup.2 (at which they do not engage in adhesion events), we adjusted the flow rate accordingly to exert shear stress from 1 to

>30 dynes/cm.sup.2. The number of leukocytes rolling in one frame of five independent fields under 200X magnification at shear stress of 0.2, 0.4, 0.8, 2.2, 4.4, 8.8, 17.6 and 26.4 dynes/cm.sup.2were quantified. A minimum of 3 experiments was performed over the entire range of shear stress and results were expressed as the mean.+-.standard deviation.

Detail Description Paragraph - DETX (185):

[0222] In these experiments, glutaraldehyde-fixed HC monolayers were prepared in 6-well plates as described above, and, where indicated, cells were pretreated with **mocarhagin** (10 g/ml) for 30 min. and washed extensively with RPMI1640 without Na+ Bicarbonate/2% FBS prior to fixation. To study P-selectin adhesive interactions, confluent CHO cells stably expressing full-length P-selectin (CHO-P) or empty vector (CHO-Mock) were released from flasks with 5 mM EDTA, washed extensively in H/H/Ca.sup.++ and resuspended at 2.times.10.sup.6/ml for utilization in the parallel-plate flow chamber. P-selectin expression on CHO-P cells was confirmed by flow cytometric analysis. Cell suspensions containing 5 mM EDTA or anti-P-selectin moAbs (10 .mu.g/ml for 30 min. on ice) were utilized as negative controls to confirm calcium-dependent binding. Cells were perfused into the chamber and allowed to fall onto cell monolayers before commencing the assessment of P-selectin adhesion at 0.2, 0.4, 0.8, and 2.2 dynes/cm.sup.2. Cellular tethering and rolling was visualized at 100.times. magnification and quantified and analyzed as described above.

Detail Description Paragraph - DETX (194):

[0231] To distinguish the contribution of PSGL-1 activity from HCELL activity on KG1a cells, enzymatic digestion of cells with OSGE or **mocarhagin,** or incubated cells with a functional blocking Ab PL-1 that both render PSGL-1 incapable of binding to L-selectin was performed (Spertini, O., Cordey, A. S., Monai, N., Giuffre, L. and Schapira, M. (1996) J. Cell Biol. 135(2), 523-531; Guyer, D. A., Moore, K. L., Lyman, E. B., Schammel, C. M. G., Rogelj, S., McEver, R. P. and Sklar, L. A. (1996) Blood 88,2415-2421; Tu. L., Chen, A., Delahunty, M. D., Moore, K. L., Watson, S. R., McEver, R. P. and Tedder, T. F. (1996) J. Immunol. 157, 3995-4004; De Luca, M., Dunlop, L. C., Andrews, R. K., Flannery, J. V., Ettling, R., Cumming, D. A., Veldman G. M. and Berndt, M. C. (1995) J. Biol. Chem. 270(45), 26734-26737 14-16). Alternatively, to distinguish the contribution of HCELL activity on KG1a cells (which is expressed exclusively on sialylated N-glycans), KG1a cells and blasts from the de novo leukemia were pretreated with neuraminidase then incubated in tunicamycin. Accordingly, L-selectin ligand activity of KG1a cells was resistant to enzymatic digestion with OSGE or **mocarhagin,** and PL-1 antibody treatments (Table 3). However, KG1a L-selectin ligand activity was eliminated following neuraminidase digestion, re-expression of ligand activity was markedly reduced following tunicamycin treatment, while ligand activity of cells treated with DMSO alone (control) returned to baseline levels (p<0.001) (Table 3). These data show that N-glycan-dependent HCELL is the primary mediator of L-selectin binding on KG1a cells. In contrast, L-selectin ligand activity of HL60 cells was completely eliminated by digestion with OSGE (p<0.001) (Table 3), and significantly inhibited following **mocarhagin** digestion (p<0.001) and by treatment with functional blocking anti-PSGl-1 PL-1 monoclonal antibody (p<0.002) (Table 3). The effectiveness of OSGE and **mocarhagin** treatments were confirmed by flow cytometric analysis of the

sensitive epitopes on CD34 and PSGL-1 with moAb QBEND-10 and moAb PSL-275, respectively. Interestingly, the fact that L-selectin ligand activity on HL60 cells was completely eliminated following OSGE digestion, but not by PL-1 moAb or **mocarhagin** treatments, suggests that HL60 cells express other non-PSGL-1, O-sialoglycoprotein L-selectin ligands. These data are consistent with previous studies demonstrating the expression of OSGE-sensitive, non-PSGL-1 L-selectin ligand(s) on HL60 cells (Ramos, C., Smith, M. J., Snapp, K. R., Kansas, G. S., Stickney, G. W., Ley, K. and Lawrence, M. B. (1998) Blood 91(3), 1067-1075).

Detail Description Paragraph - DETX (196):

[0233] Though the level of expression of PSGL-1 is equivalent between HL60 and KG1a cells, it was examined whether PSGL-1 on KG1a cells was functioning equivalently to that of PSGL-1 on HL60 cells. Since the critical N-terminal binding determinant of PSGL-1 for P-selectin overlaps with the structural binding determinant(s) for L-selectin (Snapp, K. R., Ding, H., Atkins, K., Warnke, R., Luscinskas, F. W. and Kansas, G. S. (1998) Blood 91, 154-164; De Luca, M., Dunlop, L. C., Andrews, R. K., Flannery, J. V., Ettling, R., Cumming, D. A., Veldman G. M. and Bemdt, M. C. (1995) J. Biol. Chem. 270(45), 26734-26737), it was reasoned that P-selectin binding capabilities of KG1 a and HL60 cells correlates with the efficiency of PSGL-1 binding to L-selectin. Thus, flow chamber assays of P-selectin ligand activity utilizing Chinese hamster ovary cells transfected with cDNA encoding full-length human P-selectin (CHO-P) was performed. Both HL60 and KG1a cells supported equivalent PSGL-l-mediated CHO-P cell rolling, and K562 and RPMI-8402 cells did not possess any activity (FIG. 13A). P-selectin ligand activity on KG1 a and HL60 cells was prevented following **mocarhagin** treatment (FIG. 13B). Unlike the differential capability to support L-selectin ligand activity between KG1a and HL60 cells, these data suggested that native PSGL-1 as expressed in the cell membrane was similar both structurally and functionally in these cell lines. Of note, RPMI-8402 PSGL-1 was non-functional as both an L- or P-selectin ligand, consistent with a finding that PSGL-1 on certain lymphoid cells is non-functional due to a lack of activity of .alpha.1,3 fucosyltransferases and core 2 .beta.1,6 N-acetylglucosaminyltransferases required for creation of a bioactive ligand (Vachino, G, Chang, X. -J., Veldman, G. M., Kumar, R., Sako, D., Fouser, L. A., Bemdt, M. C. and Cumming, D. A. (1995) J. Biol. Chem. 270(37), 21966-21974).

Detail Description Table CWU - DETL (3):

3TABLE 3 L-selectin Ligand Activity of KG1a and HL60 Cells Following Enzymatic or Blocking Antibody Cell Treatments under Hydrodynamic Shear Flow.sup.1 Cells and Treatments % Control Mean Lymphocyte Binding.sup.2 KG1a Cells + mocarhagin.sup.4 110.0 .+-. 10.5 +OSGE (60 .mu.g/ml).sup.5 104.2 .+-. 17.1 +PL-1 (anti-PSGL-1; 10 .mu.g/ml) 104.8 .+-. 18.0 +neuraminidase (Neur.) (0.1 0.3 .+-. 0.8* U/ml) +Neur. + DMSO 103.3 .+-. 12.3 +Neur. + Tunicamycin (15 34.3 .+-. 9.8* .mu.g/ml) HL60 Cells + **mocarhagin** 50.0 .+-. 2.0* +OSGE 12.5 .+-. 0.2* +PL-1 62.5 .+-. 1.0** Negative Controls.sup.3 <0.5 .+-. 0.3* .sup.1Using the parallel-plate flow chamber, thoracic duct lymphocytes (10 ml/ml H/H with Ca.sup.++) were perfused over glutaraldehyde-fixed monolayers of cells treated with either **m carhagin** (10 .mu.g/ml; 1 hr at 37.degree. C.), OSGE (60 .mu.g/ml; 1 hr at 37.degree. C.), PL-1 (10 .mu.g/ml;

30 min. on ice) at a defined shear stress of 4.4 dynes/cm.sup.2. .sup.2Mean lymphocyte binding from 5 fields of view from triplicate samples and a minimum of 3 experiments was divided by the mean lymphocyte binding of the untreated control cells for each respective treatment group. .sup.3Negative control groups consisted of 5 mM EDTA containing assay medium and anti-L-selectin antibody-treated (HRL-1; 10 .mu.g/ml) rat lymphocytes. .sup.4Mocarhagin digestion was verified by the inability of anti-PSGL-1 moAb PSL-275 to recognize the P-and L-selectin binding determinant or **mocarhagin**-sensitive epitope on PSGL-1 by flow cytometry. .sup.5OSGE activity was confirmed by the inability of anti-CD34 Qbend-10 to recognize its OSGE-sensitive epitope on CD34 by flow cytometry. *Statistically significant difference in lymphocyte binding compared with untreated control cells; Student's paired t-test, p < 0.001. **Statistically significant difference in lymphocyte binding compared with untreated control cells; Student's paired t-test, p < 0.002.

PGPUB-DOCUMENT-NUMBER: 20020132225

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020132225 A1

TITLE: Compositions and methods for prolonging survival of chilled platelets

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Stossel, Thomas P.	Belmont	MA	US	
Hartwig, John H.	Jamaica Plain	MA	US	
Wagner, Denisa D.	Wellesley	MA	US	

APPL-NO: 10/ 007856

DATE FILED: November 5, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60246226 20001106 US

US-CL-CURRENT: 435/4, 435/7.2

ABSTRACT:

Compositions and methods for prolonging the survival of chilled platelets are provided. The compositions include agents which inhibit the liver macrophage binding to chilled platelets.

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn.119 to U.S. Provisional Application Serial No. 60/246,226, filed Nov. 6, 2000, entitled "Compositions and Methods for Prolonging Survival of Chilled Platelets", the entire contents of which are incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (74):

[0116] One interesting platelet receptor-macrophage co-receptor pair recently identified that we believe is involved in clearance is vWfR and, .alpha.M.beta.2 (alternatively referred to in the literature as Mac-1 or CD11b/CD18) (Lopez, J. et al., Blood, Simon, d. et al., J. Exp. Med. 192). MAC1 binds to the GPIb.alpha. chain of the vWfR receptor. This interaction will immobilize leukocytes on GP1b.alpha.-coated surfaces and does not occur in

leukocytes from mice lacking Mac-1 or after treatment of platelets with **mocarhagin,** a snake venom metalloprotease that specifically removes the N-T of GP1b.alpha..

Detail Description Paragraph - DETX (153):

[0193] To further assess the role of platelet GPIb in cold-mediated clearance, this glycoprotein was removed from the external surface of human platelets with the proteolytic enzyme **mocarhagin,** and the platelets were infused into mice. Human platelets were used because **mocarhagin** does not work on mouse GPIb. Although human platelets clear rapidly from mouse circulations, the retention of enzyme-treated platelets by hepatic macrophages was 3-4 times less than of untreated platelets.

Detail Description Paragraph - DETX (171):

[0211] The a chain of the vWfR has a binding site for the .alpha.M.beta.2-integrin. We studied the adherence of cold-treated human platelets in liver sinusoids of wild type mice using intravital microscopy, and compared the adherence-ratio of sham- and **mocarhagin**- (a snake metalloproteinase, specifically cleaving the N-terminus of human GPIb.alpha.) treated platelets. These studies indicate that cold platelets adhere 3-4 times more to sinusoids than the same platelets having GPIb.alpha. removed. Hence, we believe that the .alpha.M.beta.2-GPIb.alpha. receptor pair clears chilled platelets. This observation makes .alpha.M.beta.2-GPIb.alpha. a particularly attractive receptor pair. First, the avidity of GPIb.alpha. can be modulated by the underlying cytoskeleton, providing a mechanism to transfer cold induced cytoskeletal rearrangements to the platelet surface. Cold may also promote vWf binding and potentiate clearance. Second, coldper se does not cause the removal of vWfR from the membrane surface, while activation of cells with thrombin at 37.degree. C. does, and such activated platelets are not cleared. This suggests that activation by thrombin of cold-treated platelets after rewarming might enhance their circulation and that agents which prevent the down regulation of vWfR might cause thrombin-activated platelets to be cleared.

Detail Description Paragraph - DETX (172):

[0212] To confirm the identity of vWfR as an important counterreceptor, we first block the phagocytosis of chilled platelets with specific anti-GPIb-antibodies. FIG. 14 shows the epitope map of monoclonal antibodies to GPIb.alpha., schematically represented on the extracellular domain of GPIb.alpha.. The following monoclonal antibodies are employed: AK2 (binds within the first leucine-rich repeat [amino acid residues 36-58]); AP1 and VM16d (bind to the COOH-terminal flanking and leucine-rich repeat region (201-268); SZ2 (maps to the sulfated tyrosine residues encompassing amino acids 268-281); WM23 (binds within the macroglycopeptide region of GPIb.alpha.). Alterations in glycoprotein function can be identified by differential binding of monoclonal antibodies to GPIba, binding of vWF or other proteins on resting versus chilled platelets. Furthermore, we use the A1 domain of vWf, glycocalicin and the I-domain or the lectin-binding domain of the .alpha.M.beta.2-integrin to inhibit the interaction of vWfR and the .alpha.M.beta.2-integrin. To eliminate phagocytosis induced by the Fc domain of bound IgGs, we will prepare F(ab)2's and use them as blocking agents.

Unbound IgGs and other inhibitors are removed by washing. As a second approach, GPIb.alpha. is cleaved from the surface of the human platelet using **mocarhagin**. Chilled platelets, preloaded with fluorescent dye or control platelets maintained in the warm is added to macrophages and the number of platelets ingested per macrophage determined with time of incubation. If significant inhibition of phagocytosis is measured (30-70% decrease), we isolate platelets from GPIb.alpha.-deficient animals, and perform phagocytic assays and classic circulation studies to confirm that cooled cells are still cleared.

Detail Description Table CWU - DETL (1):

2 Reagents Possible available for platelet Macrophage Knockout inhibitory receptor receptor mice studies partner References FcR family Available- IgGs IgG bound (Indik, Z. Fc.gamma.RI Ravitch to platelet et al., Fc.gamma.RIIA and FcRIIA Blood Schrieber 86: 4389- Fc.gamma.RIII (Indik, 4399) (CD16) Ibid.) CRs (C3b, Available Anti- vWfR - this (Caroll, M., C3bi) CD11/CD18 interaction Annu. Rev. CR1 Anti-GPIb can be Immunol. CR2 (mice inhibited 16: 421- CR3 and human) using 432) (.alpha.M.beta.2: Mice lacking **mocarhagin.** CD11b/ GPIb or Mac 1 CD18) EGTA CR4 RGDS (.alpha.x.beta.2) RGES Mannose Mannose (Ezekowitz, lectins R. et al., J. Exp. Med. 172: 1785- 1794) (Taylor, M., et al., J. Biol. Chem. 265: 12156- 12162) Class A AcLDL (Platt, N. et Scavenger Fucoidan, al., Proc. SR-A Poly- Natl. Acad. (acetylated inonsitol Sci., USA LDL) anti- 93: 12456- scavenger 12460) receptor IgG mAb 2FS Class B Phospho-L- PS (Savill, J. Scavenger serine et al., J. CD36 Phosphatiyl- Clin. serine Invest. 90: 1513- 1522) (Navazo, M. et al., J. Biol. Chem. 271: 15381- 15385) PS receptor vesicles Anti-CD36 receptor (monoclonal 217) CD14 (Fadok, V. et al., Nature 405: 85-90) PECAM- 1 PECAM-1 (Sun, Q. -H. et al., J. Biol. Chem. 271: 11090- 11098) Vitronectin PECAM-1 (Savill, J. (.alpha.v.beta.3) et al., Nature 343: 505- 509) (Piali, L., et al., J. Cell Biol. 130: 451- 460) SIRP.alpha. Anti-CD47 CD47 (Oldenborg, SIRPa P. -A. et knockout al., Science mice 288: 2051- 2054)

PGPUB-DOCUMENT-NUMBER: 20020127691

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020127691 A1

TITLE: Highly purified **mocarhagin, a cobra venom protease,** polynucleotides encoding same and related proteases, and therapeutic uses thereof

PUBLICATION-DATE: September 12, 2002

US-CL-CURRENT: 435/226, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

APPL-NO: 09/ 996620

DATE FILED: November 27, 2001

RELATED-US-APPL-DATA:

child 09996620 A1 20011127

parent continuation-of 09026001 19980218 US PENDING

child 09026001 19980218 US

parent continuation-in-part-of 09012637 19980123 US ABANDONED

child 09012637 19980123 US

parent continuation-in-part-of 08843373 19970415 US ABANDONED

[0001] This application is a continuation-in-part of application Ser. No. 08/012,637, filed Jan. 23, 1998, under the same title in the name of the same inventors, which was a continuation-in-part of application Ser. No. 08/843,373, filed Apr. 15, 1997.

PGPUB-DOCUMENT-NUMBER: 20020103346

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020103346 A1

TITLE: Recombinant proCVF

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Vogel, Carl-Wilhelm	Hamburg	VA	DE	
Bredehorst, Reinhard	Hamburg		DE	
Fritzinger, David	Alexandria		US	
Kock, Michael	Hamburg		DE	

APPL-NO: 09/ 925442

DATE FILED: August 10, 2001

RELATED-US-APPL-DATA:

child 09925442 A1 20010810

parent division-of 09017947 19980203 US PATENTED

child 09017947 19980203 US

parent division-of 08662227 19960614 US PATENTED

US-CL-CURRENT: 530/395, 424/186.1

ABSTRACT:

Recombinant proCVF exhibits substantially the same activity as CVF and is useful for lowering complement activity.

---------- KWIC ---------

Detail Description Paragraph - DETX (41):

[0079] Recently, **protease activities have been characterized in cobra venom** that are able to cleave human C3 into a form that resembles C3b functionally, but has a similar subunit structure to CVF1 (O'Keefe, M. C., et al, 1988, J. Biol. Chem. 263:12690). Since this activity appears to be specific, and not just a random protease, it is possible that this protease serves in the maturation pathway of CVF1. Comparing the **venom protease** cleavage sites in human C3 to the processing sites in CVF1 shows that the enzyme cleaves human C3 at a position 11 amino acid residues downstream from the actual CVF1 processing

site at the N-terminus of the .gamma.-chain, though the **venom protease** site appears to be in the middle of one of the proposed Factor B binding sites. The second **venom pr tease** cleavage site is in a position similar to the C-terminus of the .gamma.-chain, though this position has not been mapped in CVF1. The third **venom protease** cleavage site is in position 71 amino acids downstream from the N-terminus of the .beta.-chain.

Detail Description Paragraph - DETX (49):

[0087] Further, proCVF may be processed from the pre-pro-form by treatment with either whole **cobra venom or the purified proteases from cobra venom,** as described in the Doctoral thesis of M. Clare O'Keefe, Georgetown University, 1991. Thus, active proCVF may be obtained even when produced by a host incapable of the proper post-translational processing. Of course, in some expression systems proCVF will be secreted by the host even though the DNA encodes pre-proCVF.

US-PAT-NO: 6534276

DOCUMENT-IDENTIFIER: US 6534276 B1

TITLE: Methods for detecting human tissue factor inhibitor

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wun; Tze Chein	Ballwin	MO	N/A	N/A
Kretzmer; Kuniko K.	Wildwood	MO	N/A	N/A
Broze, Jr.; George J.	St. Louis	MO	N/A	N/A

APPL-NO: 09/ 627676

DATE FILED: July 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of Ser. No. 09/054,782, filed Apr. 3, 1998, as U.S. Pat. No. 6,176,587 which is a continuation of Ser. No. 08/463,323, filed Ser. No. 06/05/95, now U.S. Pat. No. 5,869,875 which is a continuation of Ser. No. 08/355,351, filed Jul. 15, 1995, abandoned, which is a continuation of Ser. No. 07/566,280, filed Aug. 13, 1990, abandoned, which is a divisional of Ser. No. 07/123,753, filed Nov. 23, 1987, issued as U.S. Pat. No. 4,966,852, which is a continuation-in-part of Ser. No. 07/077,366, filed Jul. 23, 1987, abandoned.

US-CL-CURRENT: 435/7.1, 436/536 , 436/538 , 436/545

ABSTRACT:

Molecules, such as antibodies, with binding specificity for Tissue factor Inhibitor (TFI) or for polypeptides comprising one or more Kunitz domains of TFI can be used in methods to detect TFI or polypeptides containing a Kunitz domain of TFI in biological fluids. Either direct or indirect detection methods can be carried out.

9 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Drawing Description Text - DRTX (8):

FIG. 6 shows an alignment of the basic protease inhibitor domains of TFI with other basic protease inhibitors. All the sequences except TFI were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, D.C., release 13, June 1987). 1. Bovine basic protease inhibitor precursor; 2. Bovine colostrum trypsin inhibitor; 3. Bovine serum basic protease inhibitor; 4. Edible snail isoinhibitor K; 5. Red sea turtle basic protease inhibitor (only amino acids 1-79 presented); 6. Western sand viper **venom basic protease** inhibitor I; 7. Ringhals **venom basic protease** inhibitor II; 8. Cape **cobra venom basic protease** inhibitor II; 9. Russell's viper **venom basic protease** inhibitor II; 10. Sand viper **venom basic protease** inhibitor III; 11. Eastern green mamba **venom basic protease** inhibitor I homolog; 12. Black mamba **venom basic protease** inhibitor B; 13. Black mamba **venom basic protease** inhibitor E; 14. Black mamba **venom basic protease** inhibitor I; 15. Black mamba **venom basic protease** inhibitor K; 16. .beta.-1-Bungarotoxin B chain (minor); 17. .beta.-1-Bungarotoxin B chain (major); 18. .beta.-2-Bungarotoxin B chain; 19. Horse inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123 (2)]; 20. Pig inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 21. Bovine inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 22. Human .alpha.-1-microglobulin/inter-.alpha.-trypsin inhibitor precursor [amino acids 227-283(1); 284-352(2)]; 23. TFI.[amino acids 47-117(1); 118-188(2); 210-280(3)]. Gaps were included in 16, 17, 18 to achieve best alignment. Standard one letter codes for amino acids are used.

US-PAT-NO: 6413760

DOCUMENT-IDENTIFIER: US 6413760 B1

TITLE: Highly purified **mocarhagin cobra venom protease** polynucleotides endcoding same and related proteases and therapeutic uses thereof

DATE-ISSUED: July 2, 2002

US-CL-CURRENT: 435/226, 435/252.3 , 435/320.1 , 435/325 , 536/23.1 , 536/23.2

APPL-NO: 09/ 026001

DATE FILED: February 18, 1998

PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 09/012,637, filed Jan. 23, 1998, under the same title in the name of the same inventors, now abandoned, which was a continuation-in-part of application Ser. No. 08/843,373, filed Apr. 15, 1997, now abandoned.

US-PAT-NO: 6365364

DOCUMENT-IDENTIFIER: US 6365364 B1

TITLE: Angiogenesis inhibitors and uses thereof

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mann; Kenneth G.	Grand Isle	VT	N/A	N/A
Jenny; Nancy Swords	Colchester	VT	N/A	N/A

APPL-NO: 09/ 377250

DATE FILED: August 19, 1999

PARENT-CASE:

This application claims benefit to provisional application 60/097,244 filed on Aug. 20, 1998, now abandoned. The disclosure of the 60/097,424 application is hereby incorporated by reference.

US-CL-CURRENT: 435/13, 435/184 , 435/4 , 435/7.23 , 435/7.71 , 530/380

ABSTRACT:

The present invention features isolated angiogenesis inhibitors having a molecular weight of between about 40 kDa to 50 kDa and having an amino acid sequence substantially similar to that of the amino acid sequence shown in SEQ ID NO. 2 or SEQ ID NO. 3. Further provided are methods of making and using the angiogenesis inhibitors, e.g., to inhibit vascularization or to block osteonectin and plasminogen interaction.

7 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Brief Summary Text - BSTX (19):

In one aspect, we have found that **cobra** venom, particularly from the spitting **cobra** (Naja Nigricollis Nigricollis, hereinafter Naja Nigricollis), includes a protein and particularly a protease that is especially useful for producing certain angiogenesis inhibitors. More particularly, we have found that a protease (hereinafter "K-4 **protease") found in cobra venom** specifically

cleaves plasminogen at a single site near the K4 and K5 kringles, thereby isolating, in a single fragment, nearly all of the K1-4 fragment. Disruption of the K1-4 fragment is reduced or eliminated by use of the K-4 protease. Additionally, we have found that use of the K-4 protease can enhance activity of the present angiogenesis inhibitors by removing the K5 fragment therefrom. Practice of the present invention can enhance the preparation and use of the angiogenesis inhibitors by significantly boosting yields of nearly intact K1-4 fragment.

Brief Summary Text - BSTX (67):

The K-4 protease used in the present methods can be obtained by one or a combination of strategies. For example, in one approach, the protease is isolated from a fluid obtained from the spitting **cobra** and is usually purified therefrom to enhance specific activity. In a more specific approach, the **protease is isolated from the venom of the cobra** by conventional enzyme isolation techniques such as chromatography, filtration or the like. Preferred are isolation techniques that enhance activity of a venom fraction that is capable of specifically cleaving human plasminogen between amino acid positions Pro.sup.451 and Asn.sup.452 or Asp.sup.432 depending on sequence. As noted, the specific cleavage can be determined by a variety of assays including reducing gel electrophoresis.

Brief Summary Text - BSTX (68):

A particularly preferred K-4 **protease for use in accord with the invention is isolated from venom of the cobra** and has a molecular weight of between about 5 kDa and 20 kDa and more typically about 10 kDa as determined by reducing gel electrophoresis. Especially preferred methods of isolating the Naja Nigricollis protease are described below.

US-PAT-NO: 6303754

DOCUMENT-IDENTIFIER: US 6303754 B1

TITLE: Recombinant procvf

DATE-ISSUED: October 16, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Carl-Wilhelm	Hamburg	N/A	N/A	DE
Bredehorst; Reinhard	Hamburg	N/A	N/A	DE
Fritzinger; David	Alexandria	VA	N/A	N/A
Kock; Michael	Hamburg	N/A	N/A	DE

APPL-NO: 09/ 017947

DATE FILED: February 3, 1998

PARENT-CASE:

This application is a division of application Ser. No. 08/662,227 filed on Jun. 14, 1996, now U.S. Pat. No. 5,922,320.

US-CL-CURRENT: 530/381, 424/94.64 , 435/226 , 435/69.6

ABSTRACT:

Recombinant proCVF exhibits substantially the same activity as CVF and is useful for lowering complement activity

10 Claims, 38 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 32

---------- KWIC ---------

Detailed Description Text - DETX (42):

Recently, **protease activities have been characterized in cobra venom** that are able to cleave human C3 into a form that resembles C3b functionally, but has a similar subunit structure to CVF1 (O'Keefe, M. C., et al, 1988, J. Biol. Chem. 263:12690). Since this activity appears to be specific, and not just a random protease, it is possible that this protease serves in the maturation pathway of CVF1. Comparing the **ven m protease** cleavage sites in human C3 to the processing sites in CVFI shows that the enzyme cleaves human C3 at a position 11 amino acid residues downstream from the actual CVF1 processing site

at the N-terminus of the .gamma.-chain, though the **ven m protease** site appears to be in the middle of one of the proposed Factor B binding sites. The second **venom pr tease** cleavage site is in a position similar to the C-terminus of the .gamma.-chain, though this position has not been mapped in CVF1. The third **venom protease** cleavage site is in position 71 amino acids downstream from the N-terminus of the .beta.-chain.

Detailed Description Text - DETX (50):

Further, proCVF may be processed from the pre-pro-form by treatment with either whole **cobra venom or the purified proteases from cobra venom,** as described in the Doctoral thesis of M. Clare O'Keefe, Georgetown University, 1991. Thus, active proCVF may be obtained even when produced by a host incapable of the proper post-translational processing. Of course, in some expression systems proCVF will be secreted by the host even though the DNA encodes pre-proCVF.

US-PAT-NO: 6171587

DOCUMENT-IDENTIFIER: US 6171587 B1

TITLE: Antibodies to tissue factor inhibitor

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wun; Tze Chein	Ballwin	MO	N/A	N/A
Kretzmer; Kuniko K.	Wildwood	MO	N/A	N/A
Broze, Jr.; George J.	St. Louis	MO	N/A	N/A

APPL-NO: 09/ 054782

DATE FILED: April 3, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of 08/463,323 filed Jun. 5, 1995 now U.S. Pat No. 5,849,875 which is a continuation of 08/355,351 filed Dec. 13, 1994, abandoned, which is a continuation of 07/566,280 filed Aug. 13, 1990, abandoned, which is a divisional of 07/123,753 filed Nov. 23, 1987, now U.S. Pat. No. 4,966,852, which is a continuation-in-part of 07/077,366, filed Jul. 23, 1987 now abandoned.

US-CL-CURRENT: 424/139.1, 530/387.1 , 530/387.9 , 530/388.1 , 530/389.2

ABSTRACT:

A cDNA clone having a base sequence for human tissue factor inhibitor (TFI) has been developed and characterized and the amino acid sequence of the TFI has been determined. Antibodies having a binding region specific to human tissue factor inhibitor are disclosed.

6 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Drawing Description Text - DRTX (8):

FIG. 6 shows an alignment of the basic protease inhibitor domains of TFI

with other basic protease inhibitors. All the sequences except TFI were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, D.C., release Jun. 13, 1987). 1. Bovine basic protease inhibitor precursor; 2. Bovine colostrum trypsin inhibitor; 3. Bovine serum basic protease inhibitor; 4. Edible snail isoinhibitor K; 5. Red sea turtle basic protease inhibitor (only amino acids 1-79 presented); 6. Western sand viper **venom basic protease** inhibitor I; 7. Ringhals **venom basic protease** inhibitor II; 8. Cape **cobra venom basic protease** inhibitor II; 9. Russell's viper **venom basic protease** inhibitor II; 10. Sand viper **venom basic protease** inhibitor III; 11. Eastern green mamba **venom basic protease** inhibitor I homolog; 12. Black mamba **venom basic protease** inhibitor B; 13. Black mamba **venom basic protease** inhibitor E; 14. Black mamba **venom basic protease** inhibitor I; 15. Black mamba **venom basic protease** inhibitor K; 16. .beta.-1-Bungarotoxin B chain (minor); 17. .beta.-1-Bungarotoxin B chain (major); 18. .beta.-2-Bungarotoxin B chain; 19. Horse inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123 (2)]; 20. Pig inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 21. Bovine inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 22. Human .alpha.-1-miroglobulin/inter-.alpha.-trypsin inhibitor precursor [amino acids 227-283(1); 284-352(2)]; 23. TFI [amino acids 47-117(1); 118-188(2); 210-280(3)]. Gaps were included in 16, 17, 18 to achieve best alignment. Standard one letter codes for amino acids are used.

Claims Text - CLTX (10):

3. The antibody of claim 1 which does not bind a polypeptide selected from the group consisting of bovine basic protease inhibitor precursor, bovine colostrum trypsin inhibitor, bovine serum basis protease inhibitor, edible snail isoinhibitor K, Red sea turtle basic protease inhibitor, Western sand viper **venom basic protease inhibitor I, Ringhals venom basic protease** inhibitor II, Cape **cobra venom basic protease** inhibitor II, Sand Viper **venom basic protease** inhibitor III, Eastern green mamba **venom basic protease** inhibitor I homologue, Black mamba **venom basic protease** inhibitors B, E, I, and K, .beta.-1-bungarotoxin B chain (major); .beta.-1-bungarotoxin B chain; Horse inter-.alpha.-trypsin inhibitor (amino acids 1-57 and 58-123), and Human .alpha.-1-microglobulin/inter-.alpha.-trypsin inhibitor precursor (amino acids 47-117, 118-188, and 210-280).

US-PAT-NO: 6156321

DOCUMENT-IDENTIFIER: US 6156321 A
See image for Certificate of Correction

TITLE: Tissue factor methods and compositions for coagulation and tumor treatment

DATE-ISSUED: December 5, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX	N/A	N/A
King; Steven W.	Foothill Ranch	CA	N/A	N/A
Gao; Boning	Dallas	TX	N/A	N/A

APPL-NO: 09/ 009822

DATE FILED: January 20, 1998

PARENT-CASE:

The present application is a non-provisional application and claims the benefit of provisional application Serial No. 60/042,427, filed Mar. 27, 1997; provisional application Serial No. 60/036,205, filed Jan. 27, 1997; and provisional application Serial No. 60/035,920, filed Jan. 22, 1997; the entire disclosures of each of which provisional applications are incorporated herein by reference without disclaimer.

US-CL-CURRENT: 424/198.1, 424/130.1 , 424/134.1 , 424/178.1 , 424/278.1 , 514/12 , 514/21 , 514/384 , 530/324 , 530/381

ABSTRACT:

The invention embodies the surprising discovery that Tissue Factor (TF) compositions and variants thereof specifically localize to the blood vessels within a vascularized tumor following systemic administration. The invention therefore provides methods and compositions comprising coagulant-deficient Tissue Factor for use in effecting specific coagulation and for use in tumor treatment. The TF compositions and methods of present invention may be used alone, as TF conjugates with improved half-life, or in combination with other agents, such as conventional chemotherapeutic drugs, targeted immunotoxins, targeted coaguligands, and/or in combination with Factor VIIa (FVIIa) or FVIIa activators.

47 Claims, 25 Drawing figures

Exemplary Claim Number: 1,3

Number of Drawing Sheets: 15

---------- KWIC ---------

Detailed Description Text - DETX (266):

Coagulants, such as thrombin, Factor IX/IXa, Factor X/Xa, plasmin and **metalloproteinases,** such as interstitial collagenases, stromelysins and gelatinases, also act to induce certain markers. In particular, E-selectin, **P-selectin,** PDGF and ICAM-1 are induced by thrombin (Sugamna et. al., 1992; Shankar et. al., 1994).

Detailed Description Text - DETX (334):

Russell's viper venom was shown to contain a coagulant protein by Williams and Esnouf in 1962. Kisiel (1979) isolated a venom glycoprotein that activates Factor V; and Di Scipio et al. (1977) showed that a **protease from the venom** activates human Factor X. The Factor X activator is the component contemplated for use in this invention.

US-PAT-NO: 6132729

DOCUMENT-IDENTIFIER: US 6132729 A

TITLE: Combined tissue factor and chemotherapeutic methods and compositions for coagulation and tumor treatment

DATE-ISSUED: October 17, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX	N/A	N/A
King; Steven W.	Foothill Ranch	CA	N/A	N/A
Gao; Boning	Dallas	TX	N/A	N/A

APPL-NO: 09/ 009217

DATE FILED: January 20, 1998

PARENT-CASE:

The present application is a non-provisional application and claims the benefit of provisional application Serial No. 60/042,427, filed Mar. 27, 1997; provisional application Serial No. 60/036,205, filed Jan. 27, 1997; and provisional application Serial No. 60/035,920, filed Jan. 22, 1997; the entire disclosures of each of which provisional applications are incorporated herein by reference without disclaimer.

US-CL-CURRENT: 424/198.1, 424/130.1 , 424/178.1 , 424/185.1 , 514/2 , 514/834 , 530/381 , 530/407 , 530/827 , 530/829

ABSTRACT:

The invention embodies the surprising discovery that Tissue Factor (TF) compositions and variants thereof specifically localize to the blood vessels within a vascularized tumor following systemic administration. The invention therefore provides methods and compositions comprising coagulation-deficient Tissue Factor for use in effecting specific coagulation and for use in tumor treatment. The TF compositions and methods of present invention may be used alone, as TF conjugates with improved half-life, or in combination with other agents, such as conventional chemotherapeutic drugs, targeted immunotoxins, targeted coaguligands, and/or in combination with Factor VIIa (FVIIa) or FVII activators.

46 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

---------- KWIC ---------

Detailed Description Text - DETX (274):
Coagulants, such as thrombin, Factor IX/IXa, Factor X/Xa, plasmin and **metalloproteinases,** such as interstitial collagenases, stromelysins and gelatinases, also act to induce certain markers. In particular, E-selectin, **P-selectin,** PDGF and ICAM-1 are induced by thrombin (Sugama et. al., 1992;Shankar et. al., 1994).

Detailed Description Text - DETX (344):
Russell's viper venom was shown to contain a coagulant protein by Williams and Esnouf in 1962. Kisiel (1979) isolated a venom glycoprotein that activates Factor V; and Di Scipio et al. (1977) showed that a **protease from the venom** activates human Factor X. The Factor X activator is the component contemplated for use in this invention.

US-PAT-NO: 6093399

DOCUMENT-IDENTIFIER: US 6093399 A
See image for Certificate of Correction

TITLE: Methods and compositions for the specific coagulation of vasculature

DATE-ISSUED: July 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX	N/A	N/A
Edgington; Thomas S.	La Jolla	CA	N/A	N/A

APPL-NO: 08/ 482369

DATE FILED: June 7, 1995

PARENT-CASE:

The present application is a continuation-in-part of U.S. patent application Ser. No. 08/273,567 (ABN), filed Jul. 11, 1994; which is a continuation-in-part of U.S. patent application Ser. No. 08/205,330, filed Mar. 2, 1994; which is a continuation-in-part of U.S. Ser. No. 07/846,349 (ABN), filed Mar. 5, 1992. The entire text and figures of the above-referenced disclosures are specifically incorporated herein by reference without disclaimer.

US-CL-CURRENT: 424/182.1, 424/178.1 , 424/179.1 , 424/180.1 , 530/387.1 , 530/387.3 , 530/387.7 , 530/387.9 , 530/388.1 , 530/388.22 , 530/388.85 , 530/391.7 , 530/391.9

ABSTRACT:

Disclosed are various compositions and methods for use in achieving specific blood coagulation. This is exemplified by the specific in vivo coagulation of tumor vasculature, causing tumor regression, through the site-specific delivery of a coagulant using a bispecific antibody.

103 Claims, 11 Drawing figures

Exemplary Claim Number: 1,102

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX (46):

Further inducible antigens include those inducible by a coagulant, such as by thrombin, Factor IX/IXa, Factor X/Xa, plasmin or a **metallopr teinase** (matrix **metalloproteinase,** MMP). Generally, antigens inducible by thrombin will be used. This group of antigens includes **P-selectin,** E-selectin, PDGF and ICAM-1, with the induction and targeting of **P-selectin** and/or E-selectin being generally preferred.

Detailed Description Text - DETX (78):

Coagulants, such as thrombin, Factor IX/IXa, Factor X/Xa, plasmin and **metalloproteinases,** such as interstitial collagenases, stromelysins and gelatinases, also act to induce certain markers. In particular, E-selectin, **P-selectin,** PDGF and ICAM-1 are induced by thrombin (Sugama et. al., 1992; Shankar et. al., 1994).

Detailed Description Text - DETX (146):

Russell's viper venom was shown to contain a coagulant protein by Williams and Esnouf in 1962. Kisiel (1979) isolated a venom glycoprotein that activates Factor V; and Di Scipio et al. (1977) showed that a **protease from the venom** activates human Factor X. The Factor X activator is the component contemplated for use in this invention.

US-PAT-NO: 6036955

DOCUMENT-IDENTIFIER: US 6036955 A
See image for Certificate of Correction

TITLE: Kits and methods for the specific coagulation of vasculature

DATE-ISSUED: March 14, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX	N/A	N/A
Edgington; Thomas S.	La Jolla	CA	N/A	N/A

APPL-NO: 08/ 479727

DATE FILED: June 7, 1995

PARENT-CASE:

The present application is a continuation-in-part of U.S. patent application Ser. No. 08/273,567, filed Jul. 11, 1994 (abandoned); which is a continuation-in-part of co-pending U.S. patent application Ser. No. 08/205,330, filed Mar. 2, 1994; which is a continuation-in-part of U.S. Ser. No. 07/846,349, filed Mar. 5, 1992, now abandoned. The entire text and figures of the above-referenced disclosures are specifically incorporated herein by reference without disclaimer.

US-CL-CURRENT: 424/136.1, 424/130.1 , 424/141.1 , 424/143.1 , 424/144.1 , 424/145.1 , 424/152.1 , 424/155.1 , 424/156.1 , 424/158.1 , 424/178.1 , 424/85.2 , 514/8 , 530/387.3 , 530/387.7 , 530/388.7 , 530/389.6 , 530/389.7 , 530/391.7

ABSTRACT:

Disclosed are various compositions and methods for use in achieving specific blood coagulation. This is exemplified by the specific in vivo coagulation of tumor vasculature, causing tumor regression, through the site-specific delivery of a coagulant using a bispecific antibody.

102 Claims, 11 Drawing figures

Exemplary Claim Number: 1,50

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX (44):

Further inducible antigens include those inducible by a coagulant, such as by thrombin, Factor IX/IXa, Factor X/Xa, plasmin or a **metall proteinase** (matrix **metallopr teinase,** MMP). Generally, antigens inducible by thrombin will be used. This group of antigens includes **P-selectin,** E-selectin, PDGF and ICAM-1, with the induction and targeting of **P-selectin** and/or E-selectin being generally preferred.

Detailed Description Text - DETX (76):

Coagulants, such as thrombin, Factor IX/IXa, Factor X/Xa, plasmin and **metalloproteinases,** such as interstitial collagenases, stromelysins and gelatinases, also act to induce certain markers. In particular, E-selectin, **P-selectin,** PDGF and ICAM-1 are induced by thrombin (Sugama et. al., 1992; Shankar et. al., 1994).

Detailed Description Text - DETX (142):

Russell's viper venom was shown to contain a coagulant protein by Williams and Esnouf in 1962. Kisiel (1979) isolated a venom glycoprotein that activates Factor V; and Di Scipio et al. (1977) showed that a **protease from the venom** activates human Factor X. The Factor X activator is the component contemplated for use in this invention.

US-PAT-NO: 6020181

DOCUMENT-IDENTIFIER: US 6020181 A
See image for Certificate of Correction

TITLE: Inhibition of thrombus formation by medical related apparatus comprising treating with fibrinolytic matrix metalloproteinase

DATE-ISSUED: February 1, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bini; Alessandra	New York	NY	N/A	N/A

APPL-NO: 08/ 859738

DATE FILED: May 21, 1997

PARENT-CASE:

This is a continuation-in-part of application Ser. No. 08/765,815, filed on Jan. 17, 1997, which is a continuation-in-part of application Ser. No. 08/446,887, filed on May 17, 1995, U.S. Pat. No. 5,830,468 the entire disclosures of which are incorporated herein by reference.

US-CL-CURRENT: 435/226, 435/212 , 435/219 , 604/403

ABSTRACT:

The invention provides a method of causing the degradation of fibrin(ogen) (i.e., fibrin, fibrinogen, and related substances) by means of a fibrinolytic matrix metalloproteinase, preferably an MMP-3 or MMP-7. The method of the invention can be performed in vitro to provide diagnostic information characterizing fibrin(ogen) and fibrinolytic physiology. The method can also be performed in vivo as a method of thrombolytic therapy in which a fibrinolytic matrix metalloproteinase is administered to a subject to degrade thrombus in situ. The fibrinolytic matrix metalloproteinase can be administered in conjunction with other active agents, preferably with agents having thrombolytic activity to improve thrombolytic and fibrinolytic therapy. The invention further provides compositions containing a fibrinolytic matrix metalloproteinase for the performance of fibrinolytic or thrombolytic procedures. Also provided are kits that include a fibrinolytic matrix metalloproteinase for performing fibrinolytic or thrombolytic procedures.

4 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Brief Summary Text - BSTX (12):

The matrix metalloproteinases ("MMPs" or "matrixins") are a class of enzymes that are expressed within the connective tissues of vertebrates. The MMPs occur natively in such tissues and play critical roles in the continuous processes associated with the laying down and remodeling of the extracellular matrix (ECM), hence their name: "matrix" metalloproteinases. The MMPs can be characterized as "intrinsic" or "endogenous" enzymes insofar as their proper function is within the tissues of the organism in which they are natively expressed. As such the MMPs are distinguished functionally and evolutionarily from the **metalloproteinases found in snake venom** and the like, which function outside the organism in which they are expressed, and may therefore be designated "exogenous" enzymes.

Detailed Description Text - DETX (30):

The determination of the effective amount of a composition of the invention is within the skill and discretion of the practicing clinician. Specific prophylactic or therapeutic dosages and the timing of administration can be selected depending upon prevailing conditions to achieve clinically acceptable treatment. The skilled clinician will take into account such factors as the age, sex, weight, and condition of the subject, as well as the route of administration. The skilled clinician will also recognize that the fibrinolytic activity of MMPs can be supplemented by the collateral administration (e.g., co-administration or sequential administration) of other active and/or inert substances. For example, it can be desirable to administer adjuvant agents having thrombolytic activity. These agents can have direct fibrinolytic activity or can be regulators or modulators of fibrinolysis in the system in which they are employed. For example, agents having thrombolytic activity include plasminogen activators, hirudin, enzymes (e.g., **proteases derived from snake venom**), enzyme inhibitors, anticoagulants (e.g., heparin, aspirin), antibodies (preferably monoclonal antibodies) or synthetic peptides specific for platelet gpIIb/IIIa receptor, or a combination thereof. Various such thrombolytic agents are described in Collen (1996). These agents can be administered together with or ancillary to the administration of an MMP-containing composition. Thus, such other agent or agents can be included in the MMP-containing composition, or can be administered as part of another composition.

Detailed Description Text - DETX (31):

In another embodiment, the invention includes targeted fibrinolytic matrix **metalloproteinases,** i.e., MMPs that are bound to moieties having specificity for a biological target molecule. For example, a **metalloproteinase** can be bound to an antibody by methods known in the art for attaching proteins to antibodies. In this way a **metalloproteinase** can be preferentially directed to a fibrin(ogen) substrate for improving fibrin(ogen)olytic efficacy. Thus, a fibrinolytic matrix **metalloprot inase** such as MMP-3 or MMP-7 can be linked to antibodies having specificity for fibrin or a degradation product thereof, to

platelets, specifically to **P-selectin,** to oxidized lipoproteins, etc.

US-PAT-NO: 6004555

DOCUMENT-IDENTIFIER: US 6004555 A
See image for Certificate of Correction

TITLE: Methods for the specific coagulation of vasculature

DATE-ISSUED: December 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX	N/A	N/A
Edgington; Thomas S.	La Jolla	CA	N/A	N/A

APPL-NO: 08/ 487427

DATE FILED: June 7, 1995

PARENT-CASE:

The present application is a continuation-in-part of co-pending U.S. patent application Ser. No. 08/273,567, filed Jul. 11, 1994 now abandoned; which is a continuation-in-part of co-pending U.S. patent application Ser. No. 08/205,330, filed Mar. 2, 1994; which is a continuation-in-part of U.S. Ser. No. 07/846,349, filed Mar. 5, 1992 now abandoned.

US-CL-CURRENT: 424/181.1, 424/178.1 , 424/180.1 , 435/7.23 , 530/381 , 530/382 , 530/383 , 530/384 , 530/391.7

ABSTRACT:

Disclosed are various compositions and methods for use in achieving specific blood coagulation. This is exemplified by the specific in vivo coagulation of tumor vasculature, causing tumor regression, through the site-specific delivery of a coagulant using a bispecific antibody.

87 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX (45):

Further inducible antigens include those inducible by a coagulant, such as by thrombin, Factor IX/IXa, Factor X/Xa, plasmin or a **metalloproteinase** (matrix **metalloproteinase,** MMP). Generally, antigens inducible by thrombin will be

used. This group of antigens includes **P-selectin,** E-selectin, PDGF and ICAM-1, with the induction and targeting of **P-selectin** and/or E-selectin being generally preferred.

Drawing Description Text - DRTX (86):

Coagulants, such as thrombin, Factor IX/IXa, Factor X/Xa, plasmin and **metalloproteinases,** such as interstitial collagenases, stromelysins and gelatinases, also act to induce certain markers. In particular, E-selectin, **P-selectin,** PDGF and ICAM-1 are induced by thrombin (Sugama et. al., 1992; Shankar et. al., 1994).

Drawing Description Text - DRTX (152):

Russell's viper venom was shown to contain a coagulant protein by Williams and Esnouf in 1962. Kisiel (1979) isolated a venom glycoprotein that activates Factor V; and Di Scipio et al. (1977) showed that a **protease from the venom** activates human Factor X. The Factor X activator is the component contemplated for use in this invention.

US-PAT-NO: 5922322

DOCUMENT-IDENTIFIER: US 5922322 A

TITLE: Fibrin(ogen) degradation and clot lysis by fibrinolytic matrix metalloproteinase

DATE-ISSUED: July 13, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bini; Alessandra	New York	NY	N/A	N/A

APPL-NO: 08/ 765815

DATE FILED: January 17, 1997

PARENT-CASE:

This is a continuation-in-part of application Ser. No. 08/446,887, filed on May 17, 1995, now U.S. Pat. No. 5,830,468, the entire disclosure of which is incorporated herein by reference.

US-CL-CURRENT: 424/94.67, 424/94.1 , 424/94.63 , 435/212 , 435/226 , 514/2

ABSTRACT:

The invention provides a method of causing degradation of fibrin(ogen) (i.e., fibrin, fibrinogen, and related substances) by means of a fibrinolytic metalloproteinase, preferably an endogenous metalloproteinase such as MMP-3. The method of the invention can be performed in vitro to provide diagnostic information characterizing fibrin(ogen) and fibrinolytic physiology. The method can also be performed in vivo as a method of thrombolytic therapy in which a fibrinolytic metalloproteinase is administered to a subject to degrade thrombus in situ. The endogenous fibrinolytic metalloproteinase can be administered in conjunction with other active agents, preferably with agents having thrombolytic activity to improve thrombolytic and fibrinolytic therapy. The invention further provides compositions containing a fibrinolytic metalloproteinase for the performance of fibrinolytic or thrombolytic procedures. Also provided are kits which include a fibrinolytic metalloproteinase for performing fibrinolytic or thrombolytic procedures.

30 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (21):

The determination of the effective amount of a composition of the invention is within the discretion of the skilled clinician. Specific prophylactic or therapeutic dosages and the timing of administration can be selected depending upon prevailing conditions to achieve clinically acceptable treatment. The skilled clinician will take into account such factors as the age, sex, weight, and condition of the subject, as well as the route of administration. The skilled clinician will also recognize that the fibrinolytic activity of MMPs can be enhanced by the collateral administration (e.g., co-administration or sequential administration) of other active and/or inert substances. For example, it can be desirable to administer adjunct agents having thrombolytic activity. These agents can have direct fibrinolytic activity or can be regulators or modulators of fibrinolysis in the system in which they are employed. For example, agents having thrombolytic activity include plasminogen activators, hirudin, enzymes (e.g., **proteases derived from snake venom**), enzyme inhibitors, anticoagulants (e.g., heparin, aspirin), antibodies (preferably monoclonal antibodies) or synthetic peptides specific for platelet gpIIb/IIIa receptor, or a combination thereof. Various such thrombolytic agents are described in Collen (1996). These agents can be administered together with or ancillary to the administration of an MMP-containing composition. Thus, such other agent or agents can be included in the metalloproteinase-containing composition, or can be administered as part of another composition.

Detailed Description Text - DETX (22):

In another embodiment, the invention includes targeted fibrinolytic **metalloproteinases,** i.e., **metalloproteinases** which are bound to moieties having specificity for a biological target molecule. For example, a **metalloproteinase** can be bound to an antibody by methods known in the art for attaching proteins to antibodies. In this way a **metalloproteinase** can be preferentially directed to a fibrin(ogen) substrate for improving fibrin(ogen)olytic efficacy. Thus, a fibrinolytic **metalloproteinase** such as MMP-3 can be linked to antibodies having specificity for fibrin or a degradation product thereof, to platelets, specifically to **P-selectin,** to oxidized lipoproteins, etc.

US-PAT-NO: 5922320

DOCUMENT-IDENTIFIER: US 5922320 A

TITLE: Recombinant proCVF

DATE-ISSUED: July 13, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Carl-Wilhelm	Hamburg	N/A	N/A	DE
Bredehorst; Reinhard	Hamburg	N/A	N/A	DE
Fritzinger; David	Alexandria	VA	N/A	N/A
Kock; Michael	Hamburg	N/A	N/A	DE

APPL-NO: 08/ 662227

DATE FILED: June 14, 1996

US-CL-CURRENT: 424/94.64, 435/226 , 514/12 , 514/8 , 530/380 , 530/395

ABSTRACT:

Recombinant proCVF exhibits substantially the same activity as CVF and is useful for lowering complement activity.

7 Claims, 30 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 32

---------- KWIC ---------

Detailed Description Text - DETX (45):

Recently, **protease activities have been characterized in cobra venom** that are able to cleave human C3 into a form that resembles C3b functionally, but has a similar subunit structure to CVF1 (O'Keefe, M. C., et al, 1988, J. Biol. Chem. 263:12690). Since this activity appears to be specific, and not just a random protease, it is possible that this protease serves in the maturation pathway of CVF1. Comparing the **venom protease** cleavage sites in human C3 to the processing sites in CVF1 shows that the enzyme cleaves human C3 at a position 11 amino acid residues downstream from the actual CVF1 processing site at the N-terminus of the .gamma.-chain, though the **venom protease** site appears to be in the middle of one of the proposed Factor B binding sites. The second **venom protease** cleavage site is in a position similar to the C-terminus of the .gamma.-chain, though this position has not been mapped in CVF1. The third **ven m protease** cleavage site is in position 71 amino acids downstream from the

N-terminus of the .beta.-chain.

Detailed Description Text - DETX (53):

Further, proCVF may be processed from the pre-pro-form by treatment with either whole **cobra venom or the purified proteases from cobra venom,** as described in the Doctoral thesis of M. Clare O'Keefe, Georgetown University, 1991. Thus, active proCVF may be obtained even when produced by a host incapable of the proper post-translational processing. Of course, in some expression systems proCVF will be secreted by the host even though the DNA encodes pre-proCVF.

US-PAT-NO: 5877289

DOCUMENT-IDENTIFIER: US 5877289 A
See image for Certificate of Correction

TITLE: Tissue factor compositions and ligands for the specific coagulation of vasculature

DATE-ISSUED: March 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Thorpe; Philip E.	Dallas	TX	N/A	N/A
Edgington; Thomas S.	La Jolla	CA	N/A	N/A

APPL-NO: 08/ 479733

DATE FILED: June 7, 1995

PARENT-CASE:

The present application is a continuation-in-part of co-pending U.S. patent application Ser. No. 08/273,567, filed Jul. 11, 1994; which is a continuation-in-part of U.S. patent application Ser. No. 08/205,330, filed Mar. 2, 1994, now U.S. Pat. No. 5,855,866; which is a continuation-in-part of U.S. Ser. No. 07/846,349, filed Mar. 5, 1992. The entire text and figures of the above-referenced disclosures are specifically incorporated herein by reference without disclaimer.

US-CL-CURRENT: 530/387.1, 530/381 , 530/387.3 , 530/387.7 , 530/387.9 , 530/388.1 , 530/388.22 , 530/388.85 , 530/391.7 , 530/391.9

ABSTRACT:

Disclosed are various compositions and methods for use in achieving specific blood coagulation. This is exemplified by the specific in vivo coagulation of tumor vasculature, causing tumor regression, through the site-specific delivery of a coagulant using a bispecific antibody.

100 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX (44):

Further inducible antigens include those inducible by a coagulant, such as by thrombin, Factor IX/IXa, Factor X/Xa, plasmin or a **metall pr teinase** (matrix **metallopr teinase,** MMP). Generally, antigens inducible by thrombin will be used. This group of antigens includes **P-s lectin,** E-selectin, PDGF and ICAM-1, with the induction and targeting of **P-selectin** and/or E-selectin being generally preferred.

Detailed Description Text - DETX (76):

Coagulants, such as thrombin, Factor IX/IXa, Factor X/Xa, plasmin and **metalloproteinases,** such as interstitial collagenases, stromelysins and gelatinases, also act to induce certain markers. In particular, E-selectin, **P-selectin,** PDGF and ICAM-1 are induced by thrombin (Sugama et. al., 1992; Shankar et. al., 1994).

Detailed Description Text - DETX (142):

Russell's viper venom was shown to contain a coagulant protein by Williams and Esnouf in 1962. Kisiel (1979) isolated a venom glycoprotein that activates Factor V; and Di Scipio et al. (1977) showed that a **protease from the venom** activates human Factor X. The Factor X activator is the component contemplated for use in this invention.

US-PAT-NO: 5849875

DOCUMENT-IDENTIFIER: US 5849875 A

TITLE: Human tissue factor inhibitor

DATE-ISSUED: December 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wun; Tze-Chein	St. Louis	MO	N/A	N/A
Kretzmer; Kuniko K.	Eureka	MO	N/A	N/A
Broze, Jr.; George J.	St. Louis	MO	N/A	N/A

APPL-NO: 08/ 463323

DATE FILED: June 5, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a Continuation of application Ser. No. 08/355,351 filed Dec. 13, 1994, now abandoned which is a continuation of application Ser. No. 08/093,285 filed Jul. 15, 1993, now U.S. Pat. No. 5,466,783, which is a continuation of application Ser. No. 07/566,280 filed Aug. 13, 1990, now abandoned, which is a divisional of application Ser. No. 07/123,753 filed Nov. 23, 1987, now U.S. Pat. No. 4,966,852 which is a continuation-in-part of application Ser. No. 07,077,366, filed Jul. 23, 1987, now abandoned.

US-CL-CURRENT: 530/380, 530/350 , 530/395 , 530/399

ABSTRACT:

A cDNA clone having a base sequence for human tissue factor inhibitor (TFI) has been developed and characterized and the amino acid sequence of the TFI has been determined.

8 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Drawing Description Text - DRTX (7):

FIG. 6 shows an alignment of the basic protease inhibitor domains of TFI

with other basic protease inhibitors. All the sequences except TFI were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, D.C., release 13, June 1987). 1. Bovine basic protease inhibitor precursor; 2. Bovine colostrum trypsin inhibitor; 3. Bovine serum basic protease inhibitor; 4. Edible snail isoinhibitor K; 5. Red sea turtle basic protease inhibitor (only amino acids 1-79 presented); 6. Western sand viper **venom basic protease** inhibitor I; 7. Ringhals **venom basic protease** inhibitor II; 8. Cape **cobra venom basic protease** inhibitor II; 9. Russell's viper **venom basic protease** inhibitor II; 10. Sand viper **venom basic protease** inhibitor III; 11. Eastern green mamba **venom basic protease** inhibitor I homolog; 12. Black mamba **venom basic protease** inhibitor B; 13. Black mamba **venom basic protease** inhibitor E; 14. Black mamba **venom basic protease** inhibitor I; 15. Black mamba **venom basic protease** inhibitor K; 16. .beta.-1-Bungarotoxin B chain (minor); 17. .beta.-1-Bungarotoxin B chain (major); 18. .beta.-2-Bungarotoxin B chain; 19. Horse inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123 (2)]; 20. Pig inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 21. Bovine inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 22. Human .alpha.-1-microglobulin/inter-.alpha.-trypsin inhibitor precursor [amino acids 227-283(1); 284-352(2)]; 23. TFI [amino acids 47-117(1); 118-188(2); 210-280(3)]. Gaps were included in 16, 17, 18 to achieve best alignment. Standard one letter codes for amino acids are used.

US-PAT-NO: 5773251

DOCUMENT-IDENTIFIER: US 5773251 A
See image for Certificate of Correction

TITLE: DNA clone of human tissue factor inhibitor

DATE-ISSUED: June 30, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wun; Tze-Chein	St. Louis	MO	N/A	N/A
Kretzmer; Kuniko K.	Eureka	MO	N/A	N/A
Broze, Jr.; George J.	St. Louis	MO	N/A	N/A

APPL-NO: 08/ 463602

DATE FILED: June 5, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a Continuation of application Ser. No. 08/355,351 filed Dec. 13, 1994 now abandoned, which is a continuation of application Ser. No. 08/093,285 filed Jul. 15, 1993 now abandoned, which is a continuation of application Ser. No. 07/566,280 filed Aug. 13, 1990 now abandoned, which is a divisional of application Ser. No. 07/123,753 filed Nov. 23, 1987 now U.S. Pat. No. 4,966,852, which is a continuation-in-part of application Ser. No. 07/077,366, filed Jul. 23, 1987 now abandoned.

US-CL-CURRENT: 435/69.2, 435/183 , 435/252.3 , 435/320.1 , 530/350 , 536/23.5

ABSTRACT:

A cDNA clone having a base sequence for human tissue factor inhibitor (TFI) has been developed and characterized and the amino acid sequence of the TFI has been determined.

9 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Drawing Description Text - DRTX (7):

FIG. 6 shows an alignment of the basic protease inhibitor domains of TFI with other basic protease inhibitors. All the sequences except TFI were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, D.C., release 13, June 1987). 1. Bovine basic protease inhibitor precursor; 2. Bovine colostrum trypsin inhibitor; 3. Bovine serum basic protease inhibitor; 4. Edible snail isoinhibitor K; 5. Red sea turtle basic protease inhibitor (only amino acids 1-79 presented); 6. Western sand viper **venom basic protease** inhibitor I; 7. Ringhals **venom basic protease** inhibitor II; 8. Cape **cobra venom basic protease** inhibitor II; 9. Russell's viper **venom basic protease** inhibitor II; 10. Sand viper **venom basic protease** inhibitor III; 11. Eastern green mamba **venom basic protease** inhibitor I homolog; 12. Black mamba **venom basic protease** inhibitor B; 13. Black mamba **venom basic protease** inhibitor E; 14. Black mamba **venom basic protease** inhibitor I; 15. Black mamba **venom basic protease** inhibitor K; 16. .beta.-1-Bungarotoxin B chain (minor); 17. .beta.-1-Bungarotoxin B chain (major); 18. .beta.-2-Bungarotoxin B chain; 19. Horse inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123 (2)]; 20. Pig inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 21. Bovine inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 22. Human .alpha.-1-microglobulin/inter-.alpha.-trypsin inhibitor precursor [amino acids 227-283(1); 284-352(2)]; 23. TFI [amino acids 47-117(1); 118-188(2); 210-280(3)]. Gaps were included in 16, 17, 18 to achieve best alignment. Standard one letter codes for amino acids are used.

US-PAT-NO: 5773243

DOCUMENT-IDENTIFIER: US 5773243 A

TITLE: Cobra pro CVF1

DATE-ISSUED: June 30, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fritzinger; David C.	Alexandria	VA	N/A	N/A
Bredehorst; Reinhard	Hamburg	N/A	N/A	DE
Vogel; Carl-Wilhelm	Hamburg	N/A	N/A	DE

APPL-NO: 08/ 447411

DATE FILED: May 23, 1995

PARENT-CASE:

This application is a Continuation of application Ser. No. 08/043,747, filed on Apr. 7,1993, now abandoned.

US-CL-CURRENT: 435/69.1, 530/350

ABSTRACT:

DNA sequences encoding cobra C3, CVF1, and CVF2, as well as plasmids and transformed hosts comprising such DNA sequences, are provided.

3 Claims, 41 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 29

---------- KWIC ---------

Detailed Description Text - DETX (16):

Recently, **protease activities have been characterized in cobra venom** that are able to cleave human C3 into a form that resembles C3b functionally, but has a similar subunit structure to CVF1 (O'Keefe, M. C., L. H. Caporale, and C. W. Vogel. 1988. A novel cleavage product of human complement component C3 with structural and functional properties of **cobra** venom factor. J. Biol. Chem. 263:12690). Since this activity appears to be specific, and not just a random protease, it is possible that this protease serves in the maturation pathway of CVF1. Comparing the **ven m pr tease** cleavage sites in human C3 to the processing sites in CVF1 shows that the enzyme cleaves human C3 at a

position 11 amino acid residues downstream from the actual CVF1 processing site at the N-terminus of the .gamma.-chain, though the **venom protease** site appears to be in the middle of one of the proposed Factor B binding sites. The second **venom protease** cleavage site is in a position similar to the C-terminus of the .gamma.-chain, though this position has not been mapped in CVF1. The third **venom protease** cleavage site is in position 71 amino acids downstream from the N-terminus of the .beta.-chain.

Detailed Description Text - DETX (30):

Further, C3, CVF1, and CVF2 may be processed from the pre-pro-form by treatment with either whole **cobra venom or the purified proteases from cobra venom,** as described in the Doctoral thesis of M. Clare O'Keefe, Georgetown University, 1991. Thus, active C3, CVF1, and CVF2 may be obtained even when produced by a host incapable of the proper post-translational processing.

US-PAT-NO: 5714344

DOCUMENT-IDENTIFIER: US 5714344 A

TITLE: Protease-derivatized CVF

DATE-ISSUED: February 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ollert; Markus W.	Hamburg	N/A	N/A	DE
Ziegelmuller; Patrick	Hamburg	N/A	N/A	DE
Grunwald; Thomas	Hamburg	N/A	N/A	DE
Bredehorst; Reinhard	Hamburg	N/A	N/A	DE
Vogel; Carl-Wilhelm	Hamburg	N/A	N/A	DE

APPL-NO: 08/ 118674

DATE FILED: September 10, 1993

PARENT-CASE:

This application is a Continuation-In-Part of U.S. patent application Ser. No. 08/043,747 filed Apr. 7, 1993, now abandoned which is incorporated herein by reference.

US-CL-CURRENT: 435/68.1, 435/212 , 530/350

ABSTRACT:

A novel functionally active derivative of cobra venom factor is described in which the .beta.-chain has been cleaved by treatment with a protease. Gel electrophoretic analyses of the purified derivative revealed the absence of an intact .beta.-chain and a decrease of the molecular weight.

31 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Claims Text - CLTX (2):

(a) incubating natural **cobra venom factor with a protease** for a time of 30 to 300 minutes and at a temperature of 20.degree. to 40.degree. C., to obtain said **c bra** venom factor derivative;

Claims Text - CLTX (3):
wherein said natural **cobra** venom factor is Naja naja **cobra ven m factor and said protease** is chymotrypsin.

Claims Text - CLTX (12):
(a) incubating natural **cobra venom factor with a protease** for a time of 30 to 300 minutes at a temperature of 20.degree. to 40.degree. C., to obtain said **cobra** venom factor derivative;

Claims Text - CLTX (13):
wherein said natural **cobra** venom factor is Naja naja **cobra venom factor and said protease** is chymotrypsin.

Claims Text - CLTX (22):
(a) incubating natural **cobra** venom factor having a .beta.-chain and hemolysis activity with a **protease, to obtain said cobra venom** factor derivative;

Claims Text - CLTX (23):
wherein said natural **cobra** venom factor having a .beta.-chain is Naja naja **cobra venom factor and said protease** is chymotrypsin and wherein said incubating is carried out under conditions such that said **cobra** venom factor derivative has a hemolysis activity of 50 to 97% of said hemolysis activity of said natural **cobra** venom factor and said **cobra** venom factor derivative does not contain a complete, intact .beta.-chain.

Claims Text - CLTX (32):
(a) incubating natural **cobra** venom factor having a .beta.-chain and hemolysis activity with a **protease, to obtain said cobra venom** factor derivative;

Claims Text - CLTX (33):
wherein said natural **cobra** venom factor having a .beta.-chain is Naja naja **cobra venom factor and said protease** is chymotrypsin, and said incubating is carried out such that said **cobra** venom factor derivative has a hemolysis activity of 50 to 97% of said hemolysis activity of said natural **cobra** venom factor and said **cobra** venom factor derivative does not contain a complete, intact .beta.-chain.

US-PAT-NO: 5659018

DOCUMENT-IDENTIFIER: US 5659018 A

TITLE: **Mocarhagin, a cobra venom protease,** and therapeutic uses thereof

DATE-ISSUED: August 19, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Berndt; Michael C.	Mt Eliza	N/A	N/A	AU
Dunlop; Lindsay	Kirwan	N/A	N/A	AU
Andrews; Robert	Hampton	N/A	N/A	AU
DeLuca; Mariagrazia	Dandenong North	N/A	N/A	AU

APPL-NO: 08/ 520977

DATE FILED: August 1, 1995

US-CL-CURRENT: 530/400, 435/183 , 530/350 , 530/412 , 530/413 , 530/417

ABSTRACT:

Mocarhagin, a cobra venom protease, is disclosed. Pharmaceutical compositions and therapeutic uses of the protease are also provided.

2 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

---------- KWIC ---------

Abstract Text - ABTX (1):

Mocarhagin, a cobra venom protease, is disclosed. Pharmaceutical compositions and therapeutic uses of the protease are also provided.

TITLE - TI (1):

Mocarhagin, a cobra venom protease, and therapeutic uses thereof

Brief Summary Text - BSTX (8):

The present invention provides compositions comprising a **m carhagin** protein substantially free of other cobra proteins. In preferred embodiments, the **m carhagin** protein is full-length **mocarhagin** (as described below). In other

embodiments, the **mocarhagin** protein is a fragment of full-length **mocarhagin** **having mocarhagin** proteolytic activity. Preferably, the **mocarhagin** protein is characterized by at least one characteristic selected from the group consisting of:

Brief Summary Text - BSTX (12):
(d) **mocarhagin** proteolytic activity;

Brief Summary Text - BSTX (18):
In particularly preferred embodiments, the **mocarhagin** protein is capable of cleaving PSGL-1. Compositions comprising a therapeutically effective amount of a **mocarhagin** protein and a pharmaceutically acceptable carrier are also provided.

Brief Summary Text - BSTX (19):
Methods of treating an inflammatory disease and of inhibiting selectin-mediated binding comprising administering a therapeutically effective amount of a pharmaceutical composition comprising a **mocarhagin** protein to a mammalian subject are disclosed.

Brief Summary Text - BSTX (20):
The invention also provides a method of isolating **mocarhagin** from venom, said method comprising:

Brief Summary Text - BSTX (22):
(b) eluting **mocarhagin** from said heparin affinity column. Other methods of purification of **mocarhagin** encompassed by the present invention further comprise:

Brief Summary Text - BSTX (24):
(d) eluting **mocarhagin** from said gel filtration column. Compositions comprising a protein isolated according to these methods (and optionally further comprising a pharmaceutically acceptable carrier) are also encompassed by the claimed invention. Such compositions can also be used in methods of treating an inflammatory disease and of inhibiting selectin-mediated binding which comprise administering a therapeutically effective amount of such compositions to a mammalian subject.

Brief Summary Text - BSTX (25):
Compositions comprising an antibody which specifically reacts with the **mocarhagin** or a fragment thereof having **mocarhagin** proteolytic activity are also provided.

Drawing Description Text - DRTX (2):

FIG. 1: Effect of **mocarhagin** on P-selectin binding to neutrophils. Neutrophils were pretreated for 30 min at room temperature with increasing concentrations of **mocarhagin** (circles), or with **mocarhagin** which had been treated with DFP (diisopropylfluorophosphate) (triangles).

Drawing Description Text - DRTX (3):

FIG. 2: **Protease** cleavage sites in **PSGL**-1. N-Terminal protein sequence of **PSGL**-1(SEQ. ID NO:7) indicating the cleavage sites for the signal peptidase, PACE and **mocarhagin**. The peptide that was used to raise the polyclonal antibody Rb3443 is overlined. The **mocarhagin** cleavage site for **PSGL**-1 is contrasted with the cleavage site on GP Ib.alpha. (SEQ. ID NO:8).

Drawing Description Text - DRTX (4):

FIG. 3: **Mocarhagin** digestion of soluble P-selectin glycoprotein ligand. COS conditioned medium containing [.sup.35 S]methionine-labelled sPSGL-1.T7 was untreated (lanes 1,3,5,6 and 8) or digested with 5 .mu.g/ml **mocarhagin** (lanes 2,4,7 and 9). The samples were either directly electrophoresed (lanes 1 and 2), or precipitated with the P-selectin IgG chimera LEC.gamma.1 (lanes 3 and 4), or precipitated with LEC.gamma.1 which was pretreated with **mocarhagin** (LEC.gamma.1+mo; lane 5), or immunoprecipitated with Rb3026 (lanes 6 and 7) or with Rb3443 (lanes 8 and 9).

Drawing Description Text - DRTX (6):

FIG. 5: SDS 5-20% exponential gradient polyacrylamide gel electrophoresis of purified **mocarhagin** under nonreducing (NR) and reducing (R) conditions stained with COOMASSIE BLUE. Molecular weight standards are myosin (200 kDa), .beta.-galactosidase (130 kDa), phosphorylase B (94 kDa), BSA (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and soybean trypsin inhibitor (21 kDa).

Drawing Description Text - DRTX (7):

FIG. 6: Comparison of the N-terminal amino acid sequence determined for **mocarhagin** (SEQ. ID NO:1) with **metalloprotease-disintegrins from viper venom**. Jararhagin (SEQ. ID NO:9) is a 52-kDa protease purified from Bothrops jararaca (Paine et al. (1992) J. Biol. Chem. 267, 22869-22876), HR1B (SEQ. ID NO:10) and Hr2A (SEQ. ID NO:11) are hemorrhagins derived from Trimeresurus flavoviridis (Habu) venom (Takeya et al. (1989) J. Biochem. (Tokyo) 106, 151-157; Takeya et al. (1990) J. Biol. Chem. 265, 16068-16073); the sequences for protrigramin (SEQ. ID NO:12) and pro-rhodostomin (SEQ. ID NO:13) are inferred from the cDNA sequences of the disintegrins trigramin (from T. gramineus venom (Neeper et al. (1990) Nucleic Acids Res. 18, 4255)) and rhodostomin (from Calloselasma rhodostoma (Malayan pit viper) venom (Au et al. (1991) Biochem. Biophys. Res. Commun. 181, 585-593)). Identical or conserved residues are boxed.

Detailed Description Text - DETX (2):

The present invention provides a highly specific **metalloproteinase,**

mocarhagin, which has been purified from the venom of the Mozambiquan spitting **cobra,** Naja mocambique mocambique. **M carhagin** cleaves a ten amino acid peptide from the mature N-terminus of **PSGL**-1 and abolishes the ability of **PSGL**-1 to bind **P-selectin**. These results are in accord with the negative charge/sulfated tyrosine cluster at the N-terminus of **PSGL**-1 being an important determinant of **P-selectin** recognition in addition to the recognition of carbohydrate structure.

Detailed Description Text - DETX (3):

Mocarhagin can be purified from cobra venom according to the method described in the examples below. Other methods of purifying **mocarhagin** from cobra venom will also be apparent to those skilled in the art. The progress of any purification scheme for **mocarhagin** can be monitored on the basis of the biochemical characteristics of **mocarhagin** described herein and the assays for PSGL-1 digestion and neutrophil/HL60 cell binding described below.

Detailed Description Text - DETX (4):

The major step in the preferred method of purification was heparin-affinity chromatography, based on the observation by Ogilvie & Gartner (1984) that cobra lectin-dependent hemagglutination was inhibitable by heparin. **Mocarhagin** was further purified by gel filtration on SEPHAROSE CL-6B, and had an apparent molecular weight by SDS-polyacrylamide gel electrophoresis of .about.55 kDa under nonreducing and reducing conditions (FIG. 5). Typically, 1-2 mg of purified **mocarhagin** was obtained from 0.5 g of lyophilized venom. **Mocarhagin** stained weakly with periodic acid-Schiff reagent consistent with the presence of glycosylated residues. N-Terminal sequencing of purified **mocarhagin** was achieved up to 26 residues (FIG. 6), although the initial cycles showed multiple peaks that may be suggestive of a variable N-terminus.

Detailed Description Text - DETX (5):

The proteinase requires either calcium ion or zinc ion for activity and is substantially (actually fully) inhibited by excess EDTA and by high concentrations of DFP. Pretreatment of platelets with **mocarhagin** abolishes their ability to bind the adhesive ligand, yon Willebrand Factor (vWF). This is due to proteolysis between Glu-282 and Asp-283 in the of-chain of the platelet vWF receptor, the GP Ib-V-IX complex, which occurs as the sole detectible cleavage on the intact platelet surface.

Detailed Description Text - DETX (6):

In the course of studies with vWF, it was observed that **mocarhagin** was also a potent inhibitor of **P-selectin** binding to its myeloid receptor on neutrophils. Pretreatment of either neutrophils or HL60 cells with **mocarhagin** profoundly and reproducibly affected the subsequent binding of **P-selectin** to these treated cells with an apparent IC.sub.50 (concentration of inhibitor giving 50% inhibition) of 0.1 .mu.g/ml. A representative inhibition curve from multiple studies performed with both HL60 cells and neutrophils is shown in FIG. 1. Equivalent data were obtained regardless of whether the **mocarhagin**-treated cells were washed or not washed prior to the addition of

P-selectin, i.e. whether the binding assay was actually performed in the absence or presence of the **metalloproteinase**. Further, inhibition was not reversed by incubation of the treated cells with fresh medium for up to three hours. Finally, **mocarhagin** had no effect on the molecular size of **P-selectin** or on its inherent ability to bind to myeloid cells (data not shown and see FIG. 2). Treatment of **mocarhagin** with DFP completely blocked its ability to inhibit **P-selectin** binding even if the myeloid cells were incubated with concentrations of DFP-treated **mocarhagin** up to 100 .mu.g/ml (FIG. 1), a result in accord with proteolysis of the **P-selectin** receptor. Consistent with this view, the ability of **mocarhagin** to inhibit subsequent **P-selectin** binding was divalent-cation dependent, since incubation of neutrophils with excess EDTA prior to the addition of **mocarhagin** prevented its inhibitory effect. In addition, the time course of effect of **mocarhagin** was also consistent with a proteolytic event. If cells were incubated with 12 .mu.g/ml of **mocarhagin** for ten seconds prior to the addition of EDTA and the cells then washed, **P-selectin** binding was reduced, even with this brief treatment, to 40% of normal. The concentrated supernatant from **mocarhagin** treated cells, after removal of **mocarhagin** by absorption with heparin-SEPHAROSE CL-6B, did not inhibit binding of **P-selectin** to HL60 cells indicating that a functional fragment of the **P-selectin** receptor was not released by **mocarhagin** treatment.

Detailed Description Text - DETX (7):

Although the data suggest that **mocarhagin** abolishes P-selectin binding to neutrophils by a proteolytic effect on the P-selectin receptor, cell surface labelling studies failed to identify a major substrate for **mocarhagin** on either neutrophils or HL60 cells. Both lactoperoxidase radioiodinated and periodate/tritium surface labelled neutrophils and HL60 cells were treated with **mocarhagin** and the resultant cells and supernatants compared with controls by one- and two-dimensional SDS-polyacrylamide gel analysis. With lactoperoxidase radioiodinated neutrophils and HL60 cells, no substrate or product was identified consistent with the exquisite substrate specificity of **mocarhagin** suggested by the platelet studies. With periodate-labelled cells, however, analysis under reducing conditions revealed the partial loss of a band of 40 kDa and several faint bands were evident in the supernatant. There were also increased amounts of a protein of 140 kDa in the supernatant of treated cells, although this glycoprotein band was also present in control supernatants and probably corresponds to the spontaneous shedding of small amounts of leukosialin. P-selectin has been previously established not to bind leukosialin. Since P-selectin ligand blotting assays have not identified a binding protein of 40 kDa molecular weight and since the 40-kDa glycoprotein was only partially cleaved at a concentration of **mocarhagin** much higher than that required to completely abrogate P-selectin binding (FIG. 1), it is probable that none of these minor events represents cleavage of the P-selectin receptor.

Detailed Description Text - DETX (8):

P-selectin glycoprotein-1 (PSGL-1) has recently been identified as a functional ligand for P-selectin on HL60 cells. A soluble form of PSGL-1, designated sPSGL-1.T7 (comprising amino acids 18-295 of PSGL-1), when expressed in COS cells with an 1,3/1,4 fucosyltransferase, also mediates P-selectin

binding in a calcium-dependent manner (Sako et al.). One of the striking features of PSGL-1 is its similarity to the .alpha.-chain of platelet GP Ib. Both are sialomucins and each has immediately N-terminal to the mucin core a sequence rich in negatively-charged amino acids with three potential sulfated tyrosine residues (Sako et al.; Lopez et al. (1987) Proc. Natl. Acad. Sci. USA 78, 3403; Titani et al. (1987) Proc. Natl. Acad. Sci. USA 84, 5610). Since **mocarhagin** cleaves the .alpha.-chain of GP Ib within this negative charge/sulfated tyrosine cluster (FIG. 2), we speculated that **mocarhagin** may abrogate P-selectin binding to neutrophils and HL60 cells by cleaving near the N-terminus of PSGL-1, a result which would explain the failure to identify a major substrate for **mocarhagin** on myeloid cells.

Detailed Description Text - DETX (9):

That this is indeed the case is confirmed by the data of FIG. 3. FIG. 3 shows that **mocarhagin** digestion of PACE cleaved, fucosylated sPSGL-1.T7 (comprising amino acids 42-295 of **PSGL**-1) results in only a minor shift, if any, in electrophoretic mobility of the protein on a SDS-polyacrylamide gel (lanes 1 and 2), but completely abolishes the binding of sPSGL-1.T7 to the **P-selectin** IgG chimera, LEC.gamma.1 (Sako et al.), coupled to Protein A SEPHAROSE (lanes 3 and 4). To exclude the possibility that the **protease** treatment interfered with LEC.gamma.1 binding by destroying the LEC.gamma.1 Protein A SEPHAROSE complex, the following control experiment was performed. LEC.gamma.1-Protein A SEPHAROSE beads were incubated with **mocarhagin** and then washed repeatedly to remove any residual **protease**. The **protease** treated beads were unaffected in their ability to bind sPSGL-1.T7 (lane 5). FIG. 3 also shows the reactivity of untreated and **mocarhagin** digested sPSGL-1 .T7 with two polyclonal antibodies. Rb3026 (Sako et al.), which was raised against COS produced sPSGL-1.T7, precipitates sPSGL-1 independent of **mocarhagin** digestion (lanes 6 and 7), whereas Rb3443, which was raised against the N-terminal peptide of PACE-cleaved **PSGL**-1 (QATEYEYLDYDFLPE, SEQ ID NO:2), only precipitates untreated sPSGL-1.T7 (lanes 8 and 9), indicating that the N-terminal epitope for Rb3443 is lost after **mocarhagin** digestion.

Detailed Description Text - DETX (10):

The exact **mocarhagin** cleavage site in PSGL-1 was determined by N-terminal microsequencing of purified, **mocarhagin** treated sPSGL-1.T7 protein (FIG. 2). The N-terminal sequence of **mocarhagin**-cleaved PSGL-1 was determined to be DFLPETEPPEML (SEQ ID NO:6). **Mocarhagin** removes the first ten amino acids from PACE cleaved sPSGL-1 .T7. This N-terminal peptide comprises three tyrosine residues, at least one of which is sulfated as determined by NMR. That the site of cleavage for **mocarhagin** was between Tyr-10 and Asp-11 was confirmed using the synthetic peptide, TEYEYLDYDFLPETE (SEQ ID NO :3), corresponding to residues 3-17 of mature PSGL-1. The **mocarhagin** cleavage sites on PSGL-1 and the .alpha.-chain of GP Ib are similar. Each occurs on the N-terminal site of an aspartate residue and to the C-terminal side of three potential sulfated tyrosine residues and within an overall negative charge cluster (FIG. 2). Since the proteolytic activity of **mocarhagin** is inhibited by heparin and polyanions (Ward et al., manuscript in preparation), this preference for negative charge cluster may in part explain the remarkable substrate specificity of **mocarhagin**.

Detailed Description Text - DETX (11):

Confirmation of the critical importance of the N-terminal sequence of PSGL-1 in P-selectin binding was obtained using anti-peptide antibodies. P-selectin binding to neutrophils was inhibited by 80-90% by an affinity-purified polyclonal antibody against residues Gln- 1 to Glu- 15 of mature PSGL- 1 (QATEYEYLDYDFLPE, SEQ ID NO:2), but not by an affinity purified polyclonal antibody against residues Asp-9 to Arg-23 (DYDFLPETEPPEMLR, SEQ ID NO:4) (FIG. 4) or by nonimmune rabbit IgG (not shown). This result, together with the observed sensitivity of the myeloid P-selectin receptor for **mocarhagin,** is consistent with PSGL-1 being the predominant and major receptor for P-selectin on myeloid cells.

Detailed Description Text - DETX (12):

Although P-, E-, and L-selectin all recognize similar sialated carbohydrate structures such as sialyl-Lewis x, and many glycoproteins on the surface of myeloid cells contain sialyl-Lewis x, P-selectin appears to be highly specific in its recognition of PSGL-1 (Sako et al.). The present data suggest that one cause for this specificity is the negative charge/sulfated tyrosine cluster at the N-terminus of mature PSGL-1. Proteolytic removal of a N-terminal ten amino acid peptide by **mocarhagin** abolished P-selectin binding to PSGL-1 even though this sequence (QATEYEYLDY, SEQ ID NO:5) is not glycosylated. One explanation for this phenomenon is that removal of this sequence alters the conformational integrity of PSGL-1 such that P-selectin can no longer interact with critical carbohydrate structures associated with the PSGL-1 mucin core. This is unlikely for two reasons. Firstly, an affinity purified polyclonal antibody against the N-terminal fifteen amino acids of mature PSGL-1 also strongly inhibited P-selectin binding to neutrophils. Secondly, E-selectin like P-selectin also binds to PSGL-1, but, unlike P-selectin, E-selectin binds equally well to **mocarhagin** cleaved PSGL-1 suggesting that the carbohydrate recognition structures on PSGL-1 are still inherently accessible.

Detailed Description Text - DETX (14):

For the purposes of the present invention, a protein is defined as having "**mocarhagin** proteolytic activity" when (1) it digests PSGL-1, such as in the PSGL-1 digestion assay described below, and/or (2) inhibits the binding of P-selectin to neutrophils or HL60 cells, such as in the binding inhibition assay described below. Preferably, in the PSGL-1 digestion assay complete cleavage of .sup.35 [S]-sPSGL-1.T7 is achieved in 20 min. using 10 .mu.g/ml **mocarhagin** protein; more preferably in 20 min. using less than 1 .mu.g/ml **mocarhagin** protein. Preferably, in the neutrophil/HL 60 binding inhibition assay the **mocarhagin** protein exhibits an IC.sub.50 of less than about 100 .mu.g/ml, more preferably less than about 1 .mu.g/ml.

Detailed Description Text - DETX (15):

Fragments of **mocarhagin having mocarhagin** proteolytic activity are also encompassed by the present invention. Fragments of **mocarhagin having mocarhagin** proteolytic activity can be identified by the PSGL-1 digestion assay

and neutrophil/HL60 binding inhibition assay described below. Fragments of **m carhagin** may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. For the purposes of the present invention, all references to "**mocarhagin** protein" herein include **mocarhagin** and fragments having **mocarhagin** proteolytic activity.

Detailed Description Text - DETX (16):

Isolated **mocarhagin** protein may be useful in treating conditions characterized by P- or E-selectin mediated intercellular adhesion. Such conditions include, without limitation, myocardial infarction, bacterial or viral infection, metastatic conditions, inflammatory disorders such as arthritis, acute respiratory distress syndrome, asthma, emphysema, delayed type hypersensitivity reaction, systemic lupus erythematosus, thermal injury such as burns or frostbite, autoimmune thyroiditis, experimental allergic encephalomyelitis, multiple sclerosis, multiple organ injury syndrome secondary to trauma, diabetes, Reynaud's syndrome, neutrophilic dermatosis (Sweet's syndrome), inflammatory bowel disease, Grave's disease, glomerulonephritis, gingivitis, periodontitis, hemolytic uremic syndrome, ulcerative colitis, Crohn's disease, necrotizing enterocolitis, granulocyte transfusion associated syndrome, cytokine-induced toxicity, and the like. **Mocarhagin** protein may also be useful in organ transplantation, both to prepare organs for transplantation and to quell organ transplant rejection. **Mocarhagin** protein may be used to treat hemodialysis and leukophoresis patients. **Mocarhagin** protein may be used itself as an inhibitor of P-or E-selectin-mediated intercellular adhesion or to design inhibitors of P- or E-selectin-mediated intercellular adhesion. The present invention encompasses both pharmaceutical compositions containing **mocarhagin** protein and therapeutic methods of treatment or use which employ **mocarhagin** protein.

Detailed Description Text - DETX (17):

Mocarhagin protein may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to **mocarhagin** protein and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, G-CSF, Meg-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with **mocarhagin** protein, or to minimize side effects caused by the **mocarhagin** protein. Conversely, **mocarhagin** protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic

factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

Detailed Description Text - DETX (18):

The pharmaceutical composition of the invention may be in the form of a liposome in which **mocarhagin** protein is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

Detailed Description Text - DETX (20):

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of **mocarhagin** protein is administered to a mammal having a P-selectin-mediated disease state. **Mocarhagin** protein may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, isolated **mocarhagin** protein may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering isolated **mocarhagin** protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Detailed Description Text - DETX (21):

Administration of **mocarhagin** protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

Detailed Description Text - DETX (22):

When a therapeutically effective mount of **mocarhagin** protein is administered orally, **mocarhagin** protein will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% **mocarhagin** protein, and preferably from about 25 to 90% **mocarhagin** protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil,

soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of **mocarhagin** protein and preferably from about 1 to 50% **mocarhagin** protein.

Detailed Description Text - DETX (23):

When a therapeutically effective amount of **mocarhagin** protein is administered by intravenous, cutaneous or subcutaneous injection, **mocarhagin** protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to **mocarhagin** protein an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

Detailed Description Text - DETX (24):

The amount of **mocarhagin** protein in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of **mocarhagin** protein with which to treat each individual patient. Initially, the attending physician will administer low doses of **mocarhagin** protein and observe the patient's response. Larger doses of **mocarhagin** protein may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 .mu.g to about 100 mg of **mocarhagin** protein per kg body weight.

Detailed Description Text - DETX (25):

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the **mocarhagin** protein will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Detailed Description Text - DETX (26):

M carhagin protein of the invention may also be used to immunize animals to

obtain polyclonal and monoclonal antibodies which specifically react with the **m carhagin** protein and which may inhibit P-selectin-mediated cellular adhesion. Such antibodies may be obtained using the entire **m carhagin** protein as an immunogen, or by using fragments of **mocarhagin** protein such as the soluble mature **mocarhagin** protein. Smaller fragments of the **mocarhagin** protein may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R. P. Merrifield, J. Amer. Chem. Soc. 85, 2149-2154 (1963); J. L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Detailed Description Text - DETX (33):
Purification of **mocarhagin**

Detailed Description Text - DETX (34):
The purification of **mocarhagin** from Naja mocambique mocambique venom was based on the heparin-binding properties of cobra lectins (Ogilvie & Gartner, 1984). Crude lyophilized venom (0.5 g) was solubilized in 10 mL water and loaded at 25 mL/h onto a 1.5.times.40-cm heparin-SEPHAROSE CL-6B column in TS buffer (0.01M Tris, 0.15M sodium chloride, pH 7.4) at 22 .degree. C., and washed exhaustively with TS buffer. Bound protein was eluted with a linear 250-mL 0.15-1.0M sodium chloride gradient in 0.01M Tris, pH 7.4. Fractions containing **mocarhagin** as assessed by SDS-polyacrylamide gel electrophoresis were pooled and concentrated using an Amicon ultrafiltration device fitted with a YM30 membrane, and applied at 25 mL/h onto a 1.5.times.70-cm SEPHAROSE CL-6B column equilibrated in 0.01M Tris, 0.5M sodium chloride, pH 7.4. The peak **mocarhagin** fractions were pooled and dialyzed against TS buffer. DFP-treated **mocarhagin** was prepared by incubating 250 .mu.g **mocarhagin** in 1 mL TS buffer with 8 mM (final concentration) DFP for 1 h at 22.degree. C., followed by dialysis against TS buffer.

Detailed Description Text - DETX (35):
N-terminal Sequencing of **Mocarhagin**

Detailed Description Text - DETX (39):
To examine the effect of pretreatment of neutrophils or HL60 cells with **mocarhagin** on P-selectin binding, washed cells (2.times.10.sup.7/ ml) in RPMI made 1% in fetal calf serum were incubated in the presence or absence of 10 mM EDTA followed by **mocarhagin** (0.025-100 .mu.g/ml, final concentrations) for 30 min at 22C. P-selectin binding was then either directly assessed or was assessed after centrifugation of the cells, which were then washed twice and finally resuspended in RPMI with 1% fetal calf serum. In some experiments, DFP-treated **mocarhagin** was employed in place of **mocarhagin**. To evaluate the effect of supernatant from **mocarhagin** treated cells on P-selectin binding, HL60 cells at 10.sup.8/ ml in 0.01M TRIS, 0.015M sodium chloride, 0.001M calcium chloride, pH 7.4, were incubated with **mocarhagin** (12 .mu.g/ml) for 10 min at

22.degree. C. The supernatant collected following centrifugation at 1000.times. g for 10 min was made 0.1% in BSA and loaded onto a heparin SEPHAROSE CL-6B column (0.5.times.5 cm) to remove **m carhagin**. The flow through was then tested for its effect on P-selectin binding to HL60 cells.

Detailed Description Text - DETX (40):
Effect of **mocarhagin** on surface-labelled neutrophils and HL60 cells

Detailed Description Text - DETX (41):
Washed neutrophils or HL60 cells were surface labelled by either lactoperoxidase-catalyzed radioiodination or with sodium periodate/sodium [.sup.3 H]borohydride (Berndt et al. (1981) J. Biol. Chem. 256, 59; Booth et al. (1984) J. Clin. Invest. 73, 291). Labelled cells in 0.01M Tris, 0.15M sodium chloride, 0.001M calcium chloride, pH 7.4, were incubated with **mocarhagin** (12 .mu.g/ml, final concentration) or buffer for 10 min at 22.degree. C. The cells were centrifuged at 150.times. g for 10 min, and washed twice with 0.01M Hepes, 0.15M sodium chloride, 0.001M EDTA, pH 7.4. The cells were then lysed with 1% (v/v) TRITON X-100 at 4.degree. C. for 1 h in the presence of the following protease inhibitors: diisopropyl fluorophosphate (DFP) (0.5 mM), aprotonin (10 .mu.g/ml), pepstatin (1M), leupeptin (100 .mu.g/ml) and benzamidine (10 mM). The TRITON X-100 soluble fractions separated by centrifugation at 1,000.times. g for 10 min and the supernatants from the control and **mocarhagin**-treated cells were mixed with SDS sample buffer and then electrophoresed on a 5-15% SDS-polyacrylamide gel under reducing and non-reducing conditions, or on a two dimensional nonreduced/reduced gel as described by Phillips and Agin ((1977) J. Biol. Chem. 252, 2121). The gels were then stained with COOMASSIE BRILLIANT BLUE R and either prepared for fluorography according to Bonner and Lasky ((1974) Eur. J. Biochem. 46, 83) or for autoradiography.

Detailed Description Text - DETX (42):
Mocarhagin digestion of soluble PSGL-1 (PSGL-1 Digestion Assay)

Detailed Description Text - DETX (43):
COS cells were cotransfected with three plasmids encoding soluble **PSGL**-1 (pED.sPSGL-1.T7; Sako et al.), alpha 1,3/1,4 fucosyltransferase (pEA.3/4FT) and soluble PACE (pEA-PACE SOL; Wasley et al. (1993) J. Biol. Chem. 268, 8458-8465). [.sup.35 S]Methionine-labelled COS conditioned medium containing sPSGL-1 .T7 was digested with 5 .mu.g/ml **mocarhagin** in TBS, 2 mM calcium chloride; 1 mg/ml BSA for 20 min at 37C. The ability of sPSGL-1 .T7 to bind **P-selectin** was assessed by precipitation with the **P-selectin** IgG chimera LEC.gamma.1 (Sako et al.) preabsorbed onto protein A SEPHAROSE beads in TBS, 2 mM calcium chloride, 1 mg/ml BSA for 4 h at 4C. A control experiment was also performed where the LEC.gamma.1 protein A SEPHAROSE beads were pre-treated with **mocarhagin** and then exhaustively washed prior to presentation of sPSGL-1 .T7. For immunoprecipitation analysis of untreated and **mocarhagin** treated sPSGL-1.T7, the **pr tease** was inactivated by the addition of 5 mM EDTA. sPSGL-1.T7 was then immunoprecipitated with anti-**PSGL**-1 polyclonal antibodies

Rb3026 (raised against COS produced sPSGL-1.T7; Sako et al.) or Rb3443 (raised against the N-terminal peptide of PACE cleaved **PSGL**-1: QATEYEYLDYDFLPE).

Detailed Description Text - DETX (47):
Identification of the cleavage site for **mocarhagin** on PSGL-1

Detailed Description Text - DETX (48):
20 .mu.g purified sPSGL-1.T7 was digested with 1 .mu.g **mocarhagin** in a total volume of 100 .mu.l TBS containing 2 mM CaCl.sub.2 for 1 h at 37.degree. C. The protease was inactivated by addition of 10 mM EDTA. The sample was concentrated directly onto PRO-SPIN (Applied Biosystems, Foster City, Calif.) and subjected to N-terminal sequencing on an ABI m476 gas phase protein sequencer.

Claims Text - CLTX (1):
1. An isolated **mocarhagin** protein, wherein said **mocarhagin** protein is characterized by:

Claims Text - CLTX (12):
2. A method of isolating **mocarhagin** protein of claim 1, said method comprising:

US-PAT-NO: 5466783

DOCUMENT-IDENTIFIER: US 5466783 A

TITLE: Human tissue factor inhibitor

DATE-ISSUED: November 14, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wun; Tze-Chein	St. Louis	MO	N/A	N/A
Kretzmer; Kuniko K.	Eureka	MO	N/A	N/A
Broze, Jr.; George J.	St. Louis	MO	N/A	N/A

APPL-NO: 08/ 093285

DATE FILED: July 15, 1993

PARENT-CASE:

This is a Continuation of application Ser. No. 07/566,280 filed Aug. 13, 1990, now abandoned, which is a Division of application Ser. No. 07/123,753, filed Nov. 23, 1987, which in turn is a continuation-in-part of application Ser. No. 07/077,366, filed Jul. 23, 1987, now abandoned.

US-CL-CURRENT: 530/380, 530/350 , 530/395

ABSTRACT:

A cDNA clone having a base sequence for human tissue factor inhibitor (TFI) has been developed and characterized and the amino acid sequence of the TFI has been determined.

2 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Drawing Description Text - DRTX (8):

FIG. 6 shows an alignment of the basic protease inhibitor domains of TFI with other basic protease inhibitors. All the sequences except TFI were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, D.C., release 13, Jun. 1987). 1. Bovine basic protease inhibitor precursor; 2. Bovine colostrum trypsin inhibitor; 3. Bovine serum basic protease inhibitor; 4. Edible snail

isoinhibitor K; 5. Red sea turtle basic protease inhibitor (only amino acids 1-79 presented); 6. Western sand viper **venom basic pr tease** inhibitor I; 7. Ringhals **venom basic protease** inhibitor II; 8. Cape **cobra venom basic protease** inhibitor II; 9. Russell's viper **venom basic protease** inhibitor II; 10. Sand viper **venom basic protease** inhibitor III; 11. Eastern green mamba **v n m basic protease** inhibitor I homolog; 12. Black mamba **venom basic protease** inhibitor B; 13. Black mamba **venom basic protease** inhibitor E; 14. Black mamba **venom basic protease** inhibitor I; 15. Black mamba **venom basic protease** inhibitor K; 16. .beta.-1-Bungarotoxin B chain (minor); 17. .beta.-1-Bungarotoxin B chain (major); 18. .beta.-2-Bungarotoxin B chain; 19. Horse inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123 (2)]; 20. Pig inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 21. Bovine inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 22. Human .alpha.-1-microglobulin/inter-.alpha.-trypsin inhibitor precursor [amino acids 227-283(1); 284-352(2)]; 23. TFI [amino acids 47-117(1); 118-188(2); 210-280(3)]. Gaps were included in 16, 17, 18 to achieve best alignment. Standard one letter codes for amino acids are used.

US-PAT-NO: 4966852

DOCUMENT-IDENTIFIER: US 4966852 A
See image for Certificate of Correction

TITLE: DNA clone of human tissue factor inhibitor

DATE-ISSUED: October 30, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wun; Tze-Chein	St. Louis	MO	N/A	N/A
Kretzmer; Kuniko K.	Eureka	MO	N/A	N/A
Broze, Jr.; George J.	St. Louis	MO	N/A	N/A

APPL-NO: 07/ 123753

DATE FILED: November 23, 1987

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation-in-part of copending application Ser. No. 77,366, filed July 23, 1987.

US-CL-CURRENT: 435/320.1, 435/183 , 435/69.1 , 435/69.2 , 536/23.1 , 536/23.5

ABSTRACT:

A cDNA clone having a base sequence for human tissue factor inhibitor (TFI) has been developed and characterized and the amino acid sequence of the TFI has been determined.

3 Claims, 7 Drawing figures

Exemplary Claim Number: 2

Number of Drawing Sheets: 6

---------- KWIC ---------

Drawing Description Text - DRTX (6):
FIG. 6 shows an alignment of the basic protease inhibitor domains of TFI with other basic protease inhibitors. All the sequences except TFI were obtained from the National Biomedical Research Foundation Protein Sequence Database (Georgetown University, Washington, D.C., release 13, June 1987). 1.

Bovine basic protease inhibitor precursor; 2. Bovine colostrum trypsin inhibitor; 3. Bovine serum basic protease inhibitor; 4. Edible snail isoinhibitor K; 5. Red sea turtle basic protease inhibitor (only amino acids 1-79 presented); 6. Western sand viper **venom basic protease** inhibitor I; 7. Ringhals **ven m basic protease** inhibitor II; 8. Cape **c bra venom basic pr tease** inhibitor II; 9. Russell's viper **venom basic protease** inhibitor II; 10. Sand viper **venom basic protease** inhibitor III; 11. Eastern green mamba **venom basic protease** inhibitor I homolog; 12. Black mamba **venom basic protease** inhibitor B; 13. Black mamba **venom basic protease** inhibitor E; 14. Black mamba **venom basic protease** inhibitor I; 15. Black mamba **venom basic protease** inhibitor K; 16. .beta.-1-Bungarotoxin B chain (minor); 17. .beta.-1-Bungarotoxin B chain (major); 18. .beta.-2-Bungarotoxin B chain; 19. Horse inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123 (2)]; 20. Pig inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 21. Bovine inter-.alpha.-trypsin inhibitor [amino acids 1-57(1); 58-123(2)]; 22. Human .alpha.-1-microglobulin/inter-.alpha.-trypsin inhibitor precursor [amino acids 227-283(1); 284-352(2)]; 23. TFI [amino acids 47-117(1); 118-188(2); 210-280(3)]. Gaps were included in 16, 17, 18 to achieve best alignment. Standard one letter codes for amino acids are used.